The blockade of interleukin-6 receptor as a therapeutic strategy for chronic inflammatory diseases

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Interleukin (IL)-6 is an inflammatory cytokine which plays a pathological role in chronic inflammatory disease such as Castleman's disease, rheumatoid arthritis, juvenile idiopathic arthritis and Crohn's disease. A new therapeutic strategy blocking the IL-6 signal utilizing humanized anti-IL-6 receptor antibody has been introduced for these diseases. In this review, shall describe the involvement of IL-6 in those diseases and the present state of clinical development of anti-IL-6 receptor antibody therapy.

Keywords: Castleman's disease, Crohn's disease, humanized anti-IL-6R antibody (MRA), inflammation, interleukin-6, juvenile idiopathic arthritis, rheumatoid arthritis

Cytokines play a key role in the regulation of inflammatory responses. The role of cytokines in chronic inflammatory diseases such as Castleman's disease, rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA) and Crohn's disease has been studied intensively. In these diseases, abnormal production of inflammatory cytokines leads to various clinical manifestations and laboratory findings. Interleukin (IL)-6 is one of the inflammatory cytokines involved in the pathogenesis of these diseases.

IL-6 is a pleiotropic cytokine with multiple biological activities. The cDNA of human IL-6 was cloned as a T-cell-derived factor that induces the final maturation of B-cells into immunoglobulin(Ig)-producing cells (B-cell stimulating factor [BSF]-2) [1], interferon (IFN)-β2 [2], or 26kD protein [3], independently by different groups. Furthermore, the hybridoma/plasmacytoma growth factor [4–6] and hepatocyte-stimulating factor [7] were founded to be identical with this factor. IL-6 stimulates hematopoietic stem cells to differentiate multilineage blast colony-forming cells. It also induces megakaryocytes to produce platelets and hepatocytes to synthesize acute-phase proteins such as fibrinogen, C-reactive protein (CRP) and serum amyloid A (SAA) [8]. In addition, it activates T-cells by upregulating IL-2 receptor expression and induces the proliferation of mesangial cells and epidermal keratinocytes. Furthermore, it also activates osteoclasts and differentiates neural cells.

Elevated serum concentrations of IL-6 have been observed in patients with cardiac myxoma [9], Castleman's disease [10], RA [11,12], JIA [13] and Crohn's disease [14,15].

This review shall primarily focus on new therapeutic strategies for chronic inflammatory diseases by blocking IL-6 signals utilizing a humanized anti-IL-6 receptor(R) antibody.

Humanized anti-IL-6R antibody

IL-6 functions through binding its specific receptor. This receptor consists of an 80 kDa IL-6 binding molecule termed IL-6R [16], and a 130 kDa signal transducer, glycoprotein(gp)130 [17,18]. 80 kDa IL-6R has a very short intracytoplasmic portion which lacks kinase domains for signal transduction. Once IL-6 binds to IL-6R, the complex induces homodimerization of gp130 that works as an actual signal transducing receptor for IL-6. gp130 is shared as a signal transducer by IL-6, leukemia-inhibitory factor, ciliary neurotrophic factor, oncostatin M, IL-11 and cardiotrophin-1. Therefore, the biological activities of these cytokines are overlapping. A soluble form of IL-6R (sIL-6R), lacking the transmembrane and cytoplasmic region, is present in serum and synovial fluid. Since IL-6 and sIL-6R complex can induce homodimerization of gp130 similarly to membrane IL-6R, sIL-6R can also mediate IL-6 signal. In general, other soluble forms of receptors such as tumor necrosis factor (TNF)-α and vascular endothelial growth factor (VEGF) receptor functions as antagonists against their signal transduction. In contrast, sIL-6R is capable of transducing IL-6 signal.

Upon binding with IL-6, both soluble and membrane forms of IL-6R are capable of associating with gp130 on the membrane and to mediate IL-6 signaling into the cells. IL-6 stimulation activates tyrosine kinase Janus kinase (JAK) through the cytoplasmic domain of gp130, followed by the activation of two major signal transduction

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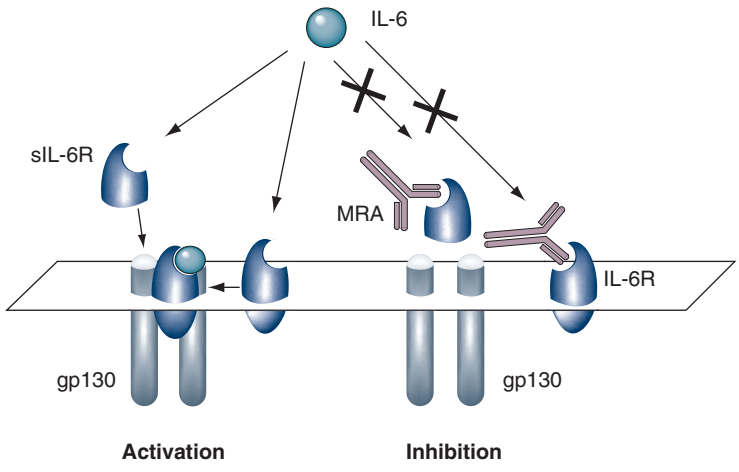
pathways – extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathway and signal transducer and activator of transcription (STAT)-3 pathway [19]. Several approaches for the blockade of IL-6 signal transduction are proposed:

- Inhibition of IL-6 production
- Neutralization of IL-6
- Blockade of IL-6 binding to IL-6R
- Blockade of IL-6/IL-6R complex binding to gp130
- Suppression of IL-6R and/or gp130 expression
- Blockade of intracytoplasmic signal from gp130 [20]

The anti-IL-6R antibody was designed as a therapeutic agent to inhibit the IL-6 signaling by blocking of IL-6 binding to IL-6R (**Figure 1**).

Murine antibodies are highly immunogenic in humans. Human antimurine antibodies are produced following repeated administration, thus resulting in allergic reactions and a decline in efficacy becoming clinical problems. Recent advances in genetic engineering technology and molecular biology have made it possible to humanize the mouse antibody in order to decrease their immunogenicity in humans. MRA is a genetically engineered monoclonal antibody (mAb) of human IgG1 subclass, formed by the technique of complementarity-determining region grafting from a murine antihuman IL-6R mAb (**Figure 2**) [21]. This humanization of the antibody decreased the immunogenicity of MRA in man and prolonged the effect when MRA was administered *in vivo*. Furthermore, MRA is capable of blocking IL-6 binding to both the membrane-bound and soluble forms of human IL-6R inhibiting the actions of IL-6. The efficacy of MRA is comparable to the parental mouse antibody in terms of the inhibitory activity of IL-6 signal transduction [21]. MRA has been developed as a therapeutic agent for chronic inflammatory diseases refractory to conventional therapies. Clinical trials of MRA are currently underway.

Figure 1. Inhibitory action of MRA in IL-6 signaling.



IL-6 binds both membrane and soluble forms of IL-6R and IL-6/IL-6R complex induces homodimerization of gp130, followed by the formation of high-affinity receptor complex of IL-6R and gp130. This association results in the activation of intracellular signal transduction. MRA blocks IL-6 binding to the membrane-bound and soluble forms of IL-6R.
gp130: Glycoprotein130; IL-6: Interleukin-6; sIL-6R: Soluble form of IL-6 receptor; MRA: Anti-IL-6 monoclonal antibody.

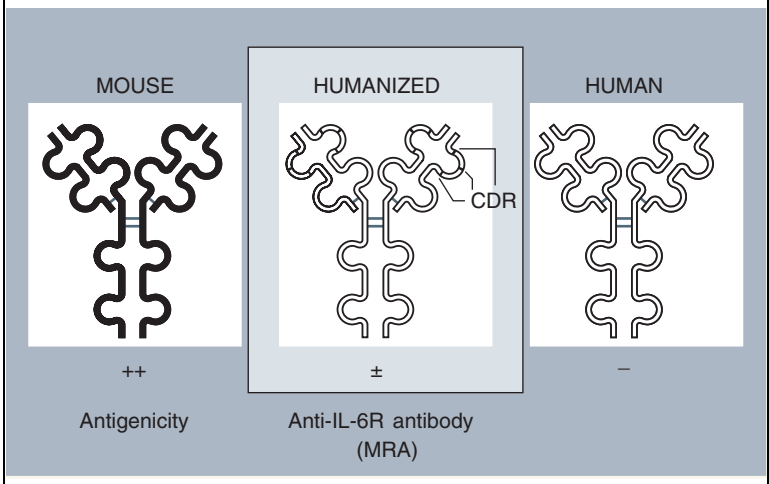
Castleman's disease

Castleman's disease & IL-6

Castleman's disease is an atypical benign lymphoproliferative disorder of unknown origin [22]. It is characterized by enlargement of lymph nodes with follicular hyperplasia and classified into two types: hyaline vascular- and plasma cell-type, according to the histological findings [23]. Clinically, it has been categorized into localized- or multicentric-type by the number of affected lymph nodes. The majority of patients with multicentric-type Castleman's disease demonstrate histological plasma cell-type. Patients with plasma cell-type frequently show systemic inflammatory symptoms and laboratory abnormalities, while little clinical manifestations are observed generally in patients with hyaline vascular-type. Physical findings in multicentric Castleman's disease are lymphadenopathy, general malaise, weight loss, anorexia, fever, hepatosplenomegaly, skin rashes and neurological disorders. Laboratory findings include elevated erythrocyte sedimentation rate (ESR), elevated CRP level, hyper-γ-globulinemia, hypoalbuminemia, thrombocytopenia, anemia and proteinuria [24]. These abnormalities appeared to be due to overproduction of IL-6.

The hypothesis that IL-6 overproduction would be a causative factor of systemic manifestations in patients with Castleman's disease was verified using IL-6 transgenic mice [25]. IL-6 transgenic mice were produced using the human IL-6 genomic gene fused with the human Igμ heavy chain enhancer. These transgenic mice showed polyclonal increase in IgG1, a massive plasmacytosis in the thymus, lymph nodes and spleen. An infiltration of plasma cells in the lung, liver and kidney, development of mesangial-proliferative glomerulonephritis and an

Figure 2. Structure of MRA.



Humanized anti-IL-6R antibody, MRA, was produced by introducing the complementarity-determining region (CDR) of murine antibody into human antibody. The humanized antibody is less immunogenic in human compared with murine antibody.
IL-6R: Interleukin-6 receptor; MRA: Anti-IL-6 monoclonal antibody.

increase in megakaryocytes in the bone marrow were also observed. These findings confirmed that IL-6 overproduction indeed causes systemic manifestations and laboratory abnormalities *in vivo*. Multicentric Castleman's disease shows similar abnormalities to those of IL-6 transgenic mice. In fact, elevated serum IL-6 concentrations are observed in patients with plasma cell-type Castleman's disease [10]. Furthermore, in patients with localized Castleman's disease, resection of the affected lymph node improves serum IL-6 levels followed by the disappearance of symptoms and biochemical abnormalities [10,26]. Conversely, the excision of one of the hyperplastic lymph nodes of patients with multicentric Castleman's disease rendered little improvements in serum IL-6 levels or clinical abnormalities [10]. High levels of IL-6 were proven to be produced from the activated B-cells present in the germinal center of the resected lymph node [10]. These findings suggest that overproduction of IL-6 may play a pathological role in plasma cell-type of Castleman's disease.

Castleman's disease treatment by MRA

Therapeutic methods for patients with multicentric Castleman's disease remain promiscuous thus far, and are often refractory to the treatment with corticosteroid and immunosuppressive agents. On the basis of the evidence that IL-6 associates with the pathophysiology of this disease, blockade of the IL-6 signal has been attempted as a new therapeutic approach to this refractory disease. Beck and colleagues reported on the treatment of a patient with localized Castleman's disease by administration of a murine anti-IL-6 mAb [26]. According to their report, the symptoms resolved and most of the abnormal laboratory values remarkably improved within a few days, however, the abnormalities returned upon cessation of antibody administration. We have explored the application of MRA to multicentric Castleman's disease [27]. We used 50 to 100 mg of MRA to treat seven patients once or twice weekly. Within 4 weeks of treatment, serum CRP levels were normalized and after 2 months of continuous treatment, anemia and lymphadenopathy improved. The treatment also reduced both the size of lymph follicles and the vascularity of germinal centers in the affected lymph node. Repeated MRA treatment was well tolerated and there was no loss of efficacy or severe adverse effects [27]. This verified that IL-6 is a key molecule in the pathogenesis of Castleman's disease and that blockade of IL-6 actions utilizing MRA is a promising method based on IL-6 biology. Phase II clinical trials are currently underway.

Rheumatoid arthritis

Rheumatoid arthritis & IL-6

RA is one of the IL-6-related chronic inflammatory diseases and characterized by the progressive joint destruction [28]. Although an autoimmune mechanism is involved in the development of RA, the pathogenic antigen epitopes are not fully understood. Intensive studies concerning the role of cytokines have revealed the importance of IL-6 as an inflammatory mediator for RA development. Collagen-induced arthritis (CIA) is an animal model mimicking human RA. Alonzi and colleagues reported that IL-6-deficient mice completely abrogate CIA, accompanied by a reduced antibody response to type II collagen [29]. Therefore, IL-6 is thought to be essential for the development of CIA [30].

Several authors have reported on the elevation of IL-6 in the serum and synovial fluid of affected joints in patients with active RA [11,12,31]. In addition, the level of IL-6 correlates with clinical activity of RA [32,33]. IL-6 is produced by proliferating synovial cells in RA patients under the influence of tumor necrosis factor (TNF)-α, IL-1β and IL-17 [34,35]. IL-6 overproduction may cause fever, general malaise, hyper-γ-globulinemia, production of rheumatoid factor, elevation of serum levels of

acute-phase proteins such as CRP, fibrinogen and SAA and hypoalbuminemia in RA patients [36]. In addition to these pro-inflammatory properties of IL-6, it is a potent inducer of T-cell differentiation and proliferation and enhances autoimmune responses. IL-6 promotes the angiogenesis essential for synovial proliferation and pannus formation through the induction of VEGF [37]. IL-6 induces bone resorption through the activation of osteoclasts [38].

Based on this evidence, anti-IL-6 therapy has been introduced into RA treatment. Takagi and colleagues were the first to test this concept when they examined the efficacy of anti-IL-6R antibody in CIA mice [39]. When antimurine IL-6R mAb was injected on the same day or 3 days after immunization with bovine type II collagen, development of arthritis was suppressed in a dose-dependent manner. However, anti-IL-6R antibody treatment did not inhibit CIA when the antibody was injected on day 7 or 14 after immunization. This observation indicates that IL-6 plays an important role in the early stage of CIA development.

MRA is specific to human IL-6R and unable to react with murine IL-6R. Therefore, the efficacy of MRA was examined for CIA in the simian model. Mihara and colleagues demonstrated the anti-arthritis effect of MRA in cynomolgus monkey with CIA [40]. MRA treatment inhibited elevation of acute-phase proteins and prevented joint destruction similarly to murine CIA. The reproduction model of synovial tissues of human RA can be obtained using the severe combined immunideficient (SCID) mouse into which human RA synovial tissues are grafted subcutaneously [41]. In this model, the intraperitoneal injection of MRA reduced the volume of the implanted tissues as well as the number of inflammatory cells, matrix metalloproteinase-9-positive cells and osteoclasts in the implanted tissues.

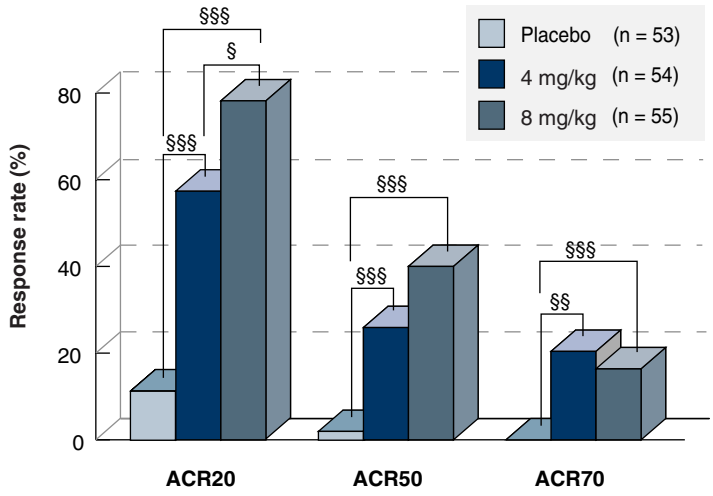
RA treatment by MRA

From 1995 to 1997, an early pilot study of MRA for severe RA patients resistant to conventional therapies was conducted. Clinical symptoms and abnormalities of laboratory findings improved significantly by the administration of 50 to 100 mg/body MRA once a week [36]. Based on this finding, Phase I clinical trials for RA patients were started in the UK 1997. Later, Phase I/II studies in Japan commenced in 1999. In the Phase I clinical trials in UK, a randomized, double-blind, placebo-controlled, dose-escalation (single dose of 0.1, 1, 5, or 10 mg/kg of MRA or placebo) trial was conducted in 45 patients with active RA [42]. Pharmacokinetics as well as the efficacy of MRA was assessed. The clinical responses were measured using the American Collage of Rheumatology (ACR) criteria. At week 2 after the administration, 55.6% of patients in the 5 mg/kg group achieved at least 20% improvement in disease activity according to the ACR criteria (an ACR20 response), while none of the placebo group achieved ACR20. However, in another dosage of MRA group, no statistically significant difference or serious adverse reaction related to MRA treatment was observed. In the Phase I/II study of MRA for RA in Japan, pharmacokinetic, safety and efficacy were assessed [43]. In an open-labeled multi-dose trial, 15 RA patients received an intravenous infusion with three doses – 2, 4, or 8 mg/kg of MRA biweekly for 6 weeks – and pharmacokinetics were assessed. Patients continued on MRA treatment for 24 weeks, and were assessed for safety, and efficacy. There was no statistically significant difference in the frequency of adverse events among the three dosage groups. In most patients, inflammatory markers such as CRP and SAA were rapidly decreased and clinical symptoms were also improved by MRA treatment.

Subsequently, a multicenter, double-blind, placebo-controlled Phase II trial for RA patients was conducted both in Japan and Europe. In Japan, 164 patients with refractory RA were randomized to receive either MRA (4 mg/kg body weight or 8 mg/kg body weight) or placebo [44]. MRA was administered intravenously every 4 weeks for a total of 3 months. The clinical responses were measured using the ACR criteria. Treatment with MRA reduced disease activity in a dose-dependent manner. At 3 months, 78% of patients in the 8 mg/kg group, 57% in the 4 mg/kg group and only 11% in the placebo group achieved an ACR20 (**Figure 3**). In Europe, a Phase II trial for RA was also performed. A total of 359 patients with active RA refractory to methotrexate treatment were randomized to receive either MRA (2 mg/kg body weight, 4 mg/kg body weight or 8 mg/kg body weight) or placebo [45]. MRA was administered intravenously every 4 weeks, either as monotherapy or in combination with methotrexate. Disease activity of patients in the 8 mg/kg group was most effectively decreased. Efficacy was also seen in 2 mg/kg and 4 mg/kg groups, particularly in combination with methotrexate.

These Phase II studies clearly demonstrate some clinical benefits of IL-6 blockade for RA [44]. However, there is a report that IL-6 increased

Figure 3. Efficacy of MRA for rheumatoid arthritis.



Patients were treated with placebo, 4mg/kg or 8mg/kg of MRA for 3 months. Percentages of responders according to indicated ACR improvement criteria are shown. Statistical analysis is performed using the two-sided chi-square test. §:$p < 0.05$; §§:$p < 0.01$; §§§:$p < 0.001$ [38].

tissue inhibitor of metalloproteinase, anti-proteases (α1-antitrypsin, α2-macrogloburin and so on) which may be a drawback in the long run. Therefore, we need to evaluate long term efficacy and safety of MRA treatment for these chronic inflammatory diseases. Currently, Phase III clinical trials are undergoing in Japan and are scheduled in Europe.

Juvenile idiopathic arthritis

Juvenile idiopathic arthritis & IL-6

Systemic JIA is a chronic inflammatory disease characterized by chronic arthritis associated with systemic features including high-spiking fever, skin rash, hepatosplenomegaly, lymphadenopathy and serositis as well as prominent laboratory evidence of inflammation [46]. Once articular involvement occurred in the course of systemic onset disease, arthritis may recurrently exacerbate and progress to polyarticular manifestation in conjunction with systemic features [47]. Arthritis and development of osteoporosis cause growth retardation and abnormal body composition. Functional outcomes of the joints of children with severe JIA are extremely poor. In addition, acute transition to macrophage-activation syndrome is life threatening [48]. Laboratory findings and disease activity are correlated with high levels of circulating IL-6 and serum IL-6 level changes with the febrile episode [49]. These observations indicate that IL-6 plays a central role in the development of systemic-onset JIA and its complications. This formidable disease is frequently refractory to various kinds of non-steroidal anti-inflammation drugs. Only high-dose corticosteroids or combination therapy using cyclosporine or methotrexate, are considered effective. However, those therapies are associated with serious adverse reactions and therefore new therapeutic strategies for this condition are urgently needed.

JIA treatment by MRA

MRA was administered to a patient with refractory systemic-onset JIA [47]. This treatment improved inflammatory markers and clinical conditions. Catch-up growth and improvement of osteoporosis were also reported. Following this case, three additional patients were treated with MRA, which brought satisfactory responses. Phase II trials have been carried out since 2002 [50]. Biweekly 2 to 8 mg/kg infusions of MRA brought improvement in clinical findings such as fever and arthritis and inflammatory markers such as CRP, ESR and SAA. Only slight adverse effects such as common cold and diarrhea were observed.

Crohn's disease

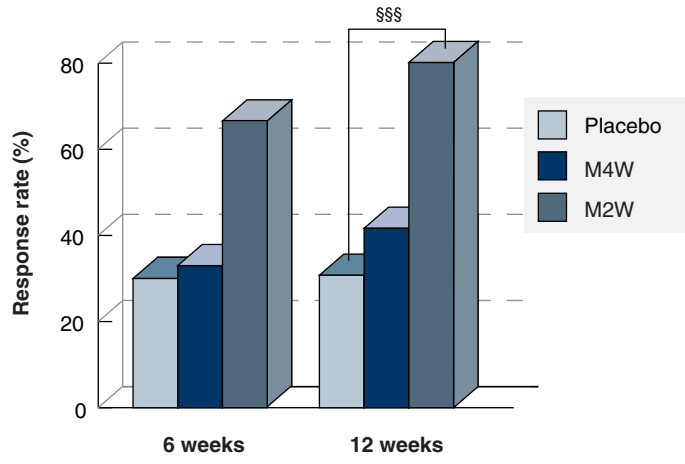
Crohn's disease & IL-6

Crohn's disease as well as ulcerative colitis are examples of chronic inflammatory bowel disease. Although both genetic and environmental factors are thought to be involved in the pathogenesis, the detailed etiology is still unknown. Accumulating evidence suggests that an inadequate and prolonged immune activation in the intestinal mucosa contributes to the development of Crohn's disease [51]. T-helper (Th)1 cytokines such as IL-1, IL-6, IL-12, IL-18, TNF-α and IFN-γ, resulting from activation of dendritic cells and macrophages, are the main modulators of the intestinal immune system in Crohn's disease. Thus, the suppression of Th1 cytokines may constitute new therapeutic approaches for Crohn's disease.

In Crohn's disease, expression of IL-6 is upregulated in intestinal lesions and correlates with the severity of endoscopically and histologically detectable inflammation [52,53]. Serum IL-6 can be a clinically relevant parameter for Crohn's disease that correlates with inflammatory activity and implies a prognostic value after steroid-induced remission [54].

The therapeutic potential of anti-IL-6R mAb has been evaluated in experimental murine colitis models. Congenic SCID mice given $CD45RB^{high}CD4^{+}$ T cells from normal mice

Figure 4. Efficacy of MRA for Crohn's disease.



Patients were treated for indicated periods with 8 mg/kg of MRA biweekly (M2W), 8mg/kg of MRA and placebo alternatively biweekly (M4W), or placebo (placebo). Bars indicate percentages of patients with more than 70 points decrease from base line Crohn's disease activity index score.
§§§: $p < 0.05$) [51].

develop Th1 cell-mediated colitis which resembles human Crohn's disease [55]. The mice also demonstrate severe wasting. Soon after the T-cell transfer, rat anti-mouse IL-6R mAb was administered intraperitoneally and continued the treatment by weekly injection for up to 8 weeks [56]. The treatment prevented the signs and symptoms of colitis and weight loss as well as the development of macroscopic and microscopic lesions of colitis. Anti-IL-6R antibody treatment induced apoptosis of intestinal lamina propria T-cells and decreased T-cell activity by downregulating the expression of adhesion molecules including intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, selectin and integrin.

Crohn's disease treatment by MRA

Based on the above observations, a rationale for anti-IL-6R antibody therapy for inflammatory bowel disease was defined and clinical studies have been initiated. A total of 36 patients with active Crohn's disease were randomly assigned to three treatment groups:

- MRA at a dose of 8 mg/kg every 2 weeks (M2W)
- 8 mg/kg of MRA and placebo alternatively every 2 weeks (M4W)
- Placebo only every 2 weeks (placebo) [57]

Patients were given the study drug for up to 12 weeks. By administration of MRA, the levels of inflammatory markers including ESR, CRP, SAA and fibrinogen were normalized and disease activity improved assessed by the Crohn's disease activity score. The clinical response rate of M2W was higher than that of M4W and placebo group (**Figure 4**). The incidence of adverse events was similar in all the groups. Therefore, anti-IL-6R antibody therapy could be a candidate therapy for Crohn's disease.

Expert opinion

We described here the pathological significance of IL-6 in chronic inflammatory diseases such as Castleman's disease RA, systemic JIA and Crohn's disease, and a present status of clinical development of MRA. IL-6 blockade utilizing MRA is promising as a therapeutic strategy for these diseases.

TNF-α inhibitors have been successfully used to treat patients with RA and Crohn's disease and the agents are about to change the therapeutic strategies for these diseases. Although there is no head-to-head trial between TNF-α inhibitors and MRA, the efficacy of MRA appeared to be similar to TNF-α inhibitors. In the treatment with MRA, there is no increase in antinuclear antibodies or antidouble strand DNA antibodies. Furthermore, MRA has an advantage that humanization of the antibody reduced the antigenecity in man and rarely induces anti-MRA antibodies. Therefore, MRA does not require the use of methotrexate or immunosuppressive agents. Further studies will be required to elucidate the difference between TNF-α inhibitors and MRA. It is also interesting to

Table 1. Clinical Development of MRA.

Target diseases	Present status (2004.3)	
Castleman's disease	Phase II Phase I	Japan USA
Rheumatoid arthritis	Phase III Phase II	Japan EU
Crohn's disease	Phase II	Japan
Systemic-onset JIA	Phase II Phase I	Japan UK
SLE	Phase I	USA
Multiple myeloma	Phase II Phase I	France USA

JIA: Juvenile idiopathic arthritis; MRA: Anti-IL-6 monoclonal antibody;
SLE: Systemic lupus erythematosus

know whether MRA is effective for patients refractory to TNF-α inhibitors.

There are other IL-6-related diseases such as systemic lupus erythematosus (SLE), psoriasis, and multiple myeloma of which pathological roles of IL-6 have been investigated. Clinical trials of SLE and multiple myeloma are currently underway in the USA and Europe. The present status of clinical development of MRA is shown in **Table 1**.

It is apparent that overproduction of IL-6 is involved in the development of these diseases, however, we do not know the causal mechanism of how IL-6 is overproduced. When the mechanism of IL-6 overproduction is elucidated, it may lead to an understanding of the exact causes of these diseases.

Outlook

Anticytokine therapy is changing the therapeutic strategy for inflammatory immunological diseases such as RA and Crohn's disease. Anti-TNF-α therapy is already established for the diseases but a considerable proportion of patients are resistant to therapy. Alternatively, a different target molecule to block for the treatment of these refractory diseases is IL-6. Humanized anti-IL-6 antibody, MRA, is a promising therapeutic agent and promises to be a new generation of anticytokine therapy in the near future.

Highlights

- Interleukin (IL)-6 is one of the pivotal cytokines involved in the pathogenesis of chronic inflammatory diseases such as Castleman's disease, rheumatoid arthritis, juvenile idiopathic arthritis, and Crohn's disease.
- Therapeutic strategies by blocking IL-6 signaling for such diseases has been developed.
- MRA is a humanized anti-IL-6 receptor antibody which specifically blocks the actions of IL-6.
- Clinical trials of MRA have shown the safety and effectiveness of anti-IL-6 therapy for refractory diseases and MRA is expected as a new generation of anticytokine therapy.

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