Mod Rheumatol (2004) 14:12–17
DOI 10.1007/s10165-003-0259-0

REVIEW ARTICLE

Shumpei Yokota · Takako Miyamae · Tomoyuki Imagawa
Naomi Iwata · Shigeki Katakura · Masaaki Mori

Inflammatory cytokines and systemic-onset juvenile idiopathic arthritis

Received: April 22, 2003 / Accepted: August 7, 2003

Abstract Systemic-onset juvenile idiopathic arthritis (JIA) is a severe and steroid-dependent disease, which sometimes progresses to the fatal disease macrophage activation syndrome. An investigation of inflammatory cytokine levels revealed increases in IL-6 in serum of systemic-onset disease patients. Continuously elevated levels of IL-6 in serum may play a important role in manifesting the clinical symptoms and signs of systemic-onset JIA, including spiking fever, rash, arthritis, and serositis. The characteristic fever spikes parallel IL-6 levels. Long-term exposure to high levels of IL-6 in children results in severe growth impairment, which was strongly suggested by the recent establishment of IL-6 transgenic mice. To avoid disease progression to macrophage activation syndrome and the adverse effects of high-dose corticosteroids, it might be reasonable to inhibit the formation of IL-6/IL-6R complex in order to block the binding to gp130 receptor, a biologically active receptor for IL-6. This review will provide evidence of the relationship between IL-6 homeostasis and systemic-onset JIA, and our recent trials of anti-IL-6R antibody (MRA) for children with acute systemic disease intractable to long-term and high-dose corticosteroid therapy. MRA could be a therapeutic modality for children with systemic-onset JIA intractable to high-dose corticosteroids.

Key words Antirheumatic drug · Cytokine · Cytokine-inducible proteins · Inflammation · Juvenile idiopathic arthritis (JIA)

S. Yokota (✉) · T. Miyamae · T. Imagawa · N. Iwata · S. Katakura · M. Mori
Department of Pediatrics, Yokohama City University School of Medicines, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
Tel. +81-45-787-2670; Fax +81-45-786-9503
e-mail: syokota@med.yokohama-cu.ac.jp

N. Iwata
Department of Rheumatic Diseases, Aichi Children's Health and Medical Center, Obu, Japan

Introduction

Arthritis in childhood is a highly heterogeneous disease. To date, several classification criteria have been developed around the world. Since diagnostic labels should be a precise and brief means of communication, and hopefully indicate the prognosis and treatment strategy for the disease, the Pediatric Standing Committee of the International League Against Rheumatism (ILAR) was established to develop standard and unified classification criteria for arthritis in childhood, which is known as juvenile idiopathic arthritis (JIA).[1] This will be a new horizon for pediatric rheumatology.

Children with systemic-onset-type JIA were described in detail by Still in 1897,[2] and the new ILAR classification criteria also include acute systemic-onset disease as one of the major types of JIA. Reports of the relative frequencies of this type of disease have varied considerably in published series, and among the seven generally less common types (10% to 20%) in Western populations.[3,4]

Acute systemic disease tends to progress to macrophage activation syndrome.[5] Recent progress in rheumatology has indicated that interleukin (IL)-6 may be the key cytokine in systemic-onset disease which forms and manifests the symptoms and signs of the disease,[6] and multiple proinflammatory cytokinemia, i.e., a cytokine storm, may be responsible for the development of macrophage activation.[7]

Efforts to treat children with severe disease by administrating various types of cytotoxic and immunosuppressive drugs have ended in failure.[3] High-dose corticosteroids is the only medication that will suppress, but not cure, the disease activity of the most severe cases. However, if it is true that IL-6 homeostasis might play an important role in developing systemic-onset disease,[8] it might be possible to modulate and correct the imbalance in the homeostasis by the use of agents designed to target solely IL-6 or IL-6 receptor (IL-6R) in order to stamp out the disease, and to halt the progression to macrophage activation syndrome. A new drug, recombinant humanized IL-6 receptor monoclonal antibody (MRA), was recently developed in Japan,

and remarkable efficacy without severe adverse effects has already been shown[9] in patients with rheumatoid arthritis,[10] Castleman's disease,[11] and adult-onset Still's disease.[12]

This review summarizes the disease features of systemic-onset JIA from the view of cytokines, and the disease transition to macrophage activation syndrome due to changes in the cytokine profiles in serum. Our experiences of the single-point hit drug MRA for children with systemic-onset JIA will be helpful to research into the critical role of cytokine in the disease transition.

Clinical features of systemic-onset JIA

Systemic-onset JIA is a systemic disease of unknown etiology, characterized by spiking fever, erythematous rash, articular involvement, and other visceral manifestations in the course of the disease. The spiking fever has a quotidian pattern, with daily temperature spikes of at least 39°C for a minimum of 2 weeks. Each febrile episode is often accompanied by an erythematous rash. Severe systemic involvement may precede the development of overt arthritis by weeks, months, or rarely years. Once articular involvement has occurred in the course of systemic-onset disease, the arthritis may recurrently exacerbate and progress to polyarticular manifestation in conjunction with systemic features. The eventual functional outcome of the joints of these children is poor, and the persistence of arthritis is a cause of permanent disability.

In addition to the characteristic fever and rash, children with systemic-onset JIA have other prominent visceral involvement, including hepatosplenomegaly, lymphadenopathy, and pericardial effusion. Hepatosplenomegaly and generalized lymphadenopathy can be prominent, and occur in most children with active systemic disease. The persistence of systemic symptoms with arthritis is a cause of abnormalities in growth and development: once the systemic disease begins, the body height of the affected children is usually recorded as being steady during the course of the disease, even 5–10 years after onset. Moreover, acute progression to macrophage activation syndrome is associated with serious morbidity and sometimes death.[13]

Specific serum features of systemic-onset JIA are lacking, and both rheumatoid factor and antinuclear antibody are essentially negative. Remarkable increases in acute-phase reactants are common. Erythrocyte sedimentation rate and C-reactive protein are useful measures of active disease at onset and during follow-up of a child with systemic-onset disease. Leukocytosis is common, and may be a reliable monitor of the active disease. Leukocyte counts are strikingly high, usually 15000–30000/μl, and most of them are segmented neutrophils but not immature myelocytes or even stabbed cells. The platelet count usually rises dramatically in active disease, and the rapid development of thrombocytosis may be a signal of exacerbation of the disease. These findings indicate that the basic setting of systemic-onset JIA is a severe inflammatory process. Progressive anemia may also be attributable to chronic inflammation, and hypergammaglobulinemia develops, accompanied by persistent inflammation. The active inflammatory clinical state, the increased levels of acute-phase reactants, and the induction of leukocytes from bone marrow are attributable to elevated levels of inflammatory cytokines, in particular levels of IL-6 along with other cytokines.

Macrophage activation syndrome and disease transition

Macrophage activation syndrome is a disease which occurs after transition from systemic-onset JIA, and is presumably associated with the rapid production of massive and various pro-inflammatory cytokines due to the activation of macrophages and T-cells by unknown triggering factor(s).[13] It was associated with serious morbidity and death until the introduction of intravenous methylprednisolone pulse therapy and cyclosporine as the resolution therapy.[14] The rapid development of persistent fever, serositis, hepatosplenomegaly, and a bleeding tendency is characteristic. Leukopenia, thrombocytopenia, and progressively elevated enzyme concentrations of aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase are present. Erythrocyte sedimentation rate and C-reactive protein are paradoxically low in association with hypofibrinogenemia induced by consumptive coagulopathy and disseminated intravascular coagulation. Active systemic disease is associated with elevated levels of fibrin degradation products, fibrin-derived product-E, and D-dimer. The disease ends in renal failure, pancreatic enzyme activation, respiratory distress syndrome, and finally endothelial permeability dysfunction.

Serum ferritin levels are slightly elevated by a few hundred nanograms per millilitre in children with active systemic-onset JIA, and the rapid elevation from moderate to extremely high levels of more than tens of thousands of nanograms per millilitre may indicate the disease transition from systemic-onset disease to macrophage activation syndrome. Serum ferritin is a carrier/reservoir protein of iron, which is known to be one of the cytokine-inducible proteins, and to be increased by TNF-α stimulation.[15] Thus, the remarkably increased levels of serum ferritin indicate that the disease transition from systemic-onset disease to macrophage activation syndrome may be at least partly related to the massive production of TNF-α.

Urinary β-2 microglobulin levels are another reliable measure of active systemic-onset JIA and the disease transition to macrophage activation syndrome. β-2 microglobulin is a component of HLA class I molecule,[16] and also one of the cytokine-inducible proteins. During the inflammatory process required for the expression of HLA class I molecule, the transcription and translation of β-2 microglobulin is activated to produce complete molecules of HLA class I, and to express them on the cell surface by interferon (IFN)-γ stimulation.[17]

Increased levels of aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase in macrophage

Table 1. Cytokine-inducible proteins

Cytokine-inducible protein	Cytokine
Ferritin	TNF-α
β2-microglobulin	IFN-γ
Neopterin	IFNs
2′-5′-Oligoadenylate synthetase	IFNs

TNF, tumor necrosis factor; IFN, interferon

activation syndrome indicate systemic tissue damage or cell death, presumably due to the apoptotic effects of TNF-α. The binding of TNF-α to their receptors leads to the induction of at least two signal transduction pathways: the NF-κB activation pathway and the caspase activation pathway.[18] The former leads to the regulation of the transcription of various proteins, and the latter leads to the mitochondrial permeability transition.[19] Cyclosporine, but not tacrolimus, has been shown to block the mitochondrial permeability transition by TNF-α signaling,[20] and within about 2 days after the administration of cyclosporine along with corticosteroids, elevated levels of serum aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase decrease dramatically.

Other serum proteins, i.e., neopterin[21] and 2′-5′-adenyl synthetase,[22] are also IFN-inducible proteins, and the increased levels of these proteins in serum strongly suggest hypercytokinemia of IFNs. Thus, the determination of serum ferritin, neopterin, 2′-5′-oligoadenylate synthetase, and urinary β-2 microglobulin is the most important bedside evaluation when estimating the level of active systemic disease and macrophage activation syndrome (Table 1).

Since IL-6 is the major cytokine detectable in serum from systemic-onset disease, and various kinds of proinflammatory cytokines in serum are a characteristic feature of macrophage activation syndrome, in particular levels of IFN-γ and TNF-α along with a number of other cytokines including M-CSF, IL-2, IL-1, and IL-6, the so-called cytokine storm,[13] the disease transition from active systemic disease to macrophage activation syndrome is attributable to the changes in the cytokine pattern of each disease condition.

Corticosteroid dependency and adverse events in the treatment of systemic-onset JIA

The treatment of children with severe systemic-onset JIA has usually ended in failure. Although children who have made the transition to macrophage activation syndrome have recently be treated successfully in our clinic[6] with a combination of corticosteroids, including intravenous methylprednisolone pulses, cyclosporine, and sometimes plasma exchanges to remove overproduced proinflammatory cytokines, the active disease is frequently refractory to various kinds of nonsteroidal anti-inflammatory, cytotoxic, and immunosuppressive drugs. Only high-dose corticosteroids, or their combinatorial use with cyclosporine or methotrexate, are considered to be effective for spiking fever, characteristic erythematous rash, articular involvement, and visceral manifestations.

Nevertheless, the acute-phase reactant levels commonly fluctuate. Most children with severe active disease are apt to have continuously high levels of erythrocyte sedimentation rate and C-reactive protein, which indicate inadequate suppression of disease activity, poor prognosis, and sometimes disease transition to macrophage activation syndrome. Moreover, the prolonged use of systemic high-dose corticosteroids, or even steroid pulse therapy, leads to iatrogenic Cushing-like syndrome, growth impairment, bone fractures, cataracts, and increased susceptibility to overwhelming infection.

Disease manifestation and laboratory findings from the viewpoint of pro-inflammatory cytokines

IL-6 functions as a hepatocyte-stimulating factor and induces the expression of various acute-phase proteins, including C-reactive protein, serum amyloid A, and fibrinogen, through gp130 signals.[23] IL-6 is also a pyrogenic cytokine, and IL-6 levels parallel the fever spikes.[24] The hypothalamus, the center of body temperature regulation, is one of the targets of the endogenous pyrogens IL-1 and IL-6, which induce the production of prostaglandin to stimulate the hypothalamus.[25] In bone marrow, IL-6 plays an important role in the signal from gp130 in hematopoietic progenitor cell expansion.[26] Thus, it might be possible to explain clinical and laboratory features of systemic-onset JIA by elevated levels of circulating proinflammatory cytokines, and in particular levels of IL-6.

Children with early-onset and long-duration systemic disease have abnormalities in growth and development. Although the long-term and high-dose use of corticosteroids would be partly responsible for the growth failure in this disease, the experimental evidence shows that MUP/hIL-6 transgenic mice over-expressing human IL-6 are growth-retarded,[27] and that the increased expression of suppressors of cytokine signaling genes in MUP/hIL-6 mice inhibits the activation of transcriptional factor STAT-5 by growth hormone, eventually resulting in growth failure,[28] and indicates that continuously over-produced circulating IL-6 in vivo is a causative agent for growth and developmental impairment. Further, the growth impairment of human IL-6 transgenic mice was completely abolished by the neutralization of IL-6. In the IL-6 and IL-6R transgenic mice that have constitutively activated gp130, hematological disorders such as thrombocytosis, hypergammaglobulinemia, and lymphoid infiltration in non-lymphoid organs have been observed.[24] Taken together, most clinical manifestations and inflammatory laboratory features could be attributable to the constitutively increased levels of circulating IL-6.

Previous reports have indicated the close relationship between increased IL-6 levels and systemic-onset disease. De Benedetti et al.[29] showed the correlation of serum IL-6

levels with joint involvement and thrombocytosis, and also high levels of IL-6/sIL-6R complexes in systemic-onset JIA.[30] Serum IL-6 levels in children with systemic-onset JIA were correlated with disease activity and with the extent and severity of joint involvement.[31] Woo and co-workers[32] showed that there is good correlation between laboratory measures of disease activity, C-reactive protein, erythrocyte sedimentation rate, and clinical scores for disease activity, which are further correlated with high levels of circulating IL-6, and that serum IL-6 levels rise and fall with the febrile episodes. Recently, after finding evidence that the levels of soluble IL-6R are significantly increased in the course of a febrile episode, while levels of antagonists to IL-6 are not changed in the face of increased levels of IL-6 in systemic-onset JIA, Woo and co-workers[33] hypothesized that an imbalance of IL-6 homeostasis is important in the pathogenesis of this disease. These observations strongly indicate that IL-6 and IL-6R might play a central role in the induction and progression of systemic-onset JIA and its complications.

Biologic characteristics of IL-6, IL-6 receptor, and gp130

IL-6 was originally identified as a B-cell differentiation factor, but it is now known to be a pleiotropic cytokine that regulates the immune response, haematopoiesis, the acute phase response, and inflammation,[34] and has been implicated in several pathologic conditions, including inflammatory, autoimmune, and malignant diseases.[35] IL-6 mediates its functions through two membrane proteins: IL-6R, an 80-kD ligand-binding receptor, and gp130, a 130-kD signal transducing element. A soluble form of IL-6R, lacking the transmembrane and cytoplasmic regions, is present in serum. The gp130 is shared among the receptors for IL-6, leukemia inhibitory factor, ciliary neutrophilic factor, oncostatin M, IL-11, and cardiotrophin-1 as a component which is critical for signal transduction.[36] The sharing of receptor subunits is one of the mechanisms through which the functional redundancy of IL-6 activities occurs.

The gp130 receptor has no IL-6 binding capability by itself. Upon binding with IL-6, both soluble and membrane IL-6R is able to associate with gp130 on the membrane and to mediate intracellular signaling.[37] IL-6 stimulation activates JAK tyrosine kinases, which are constitutively associated with gp130, leading to the induction of two major signal transduction pathways, the ERK MARK pathway and the STAT-3 pathway, through the cytoplasmic domain of gp130.[38] Recently, a negative regulation system of gp130 signals, in particular STAT-3 signals, was discovered, and termed a "suppressor of cytokine signaling" (SOCS).[39] The constitutively high levels of IL-6/IL-6R complexes in disease conditions such as systemic-onset JIA may induce intracellular signaling as well as a negative feedback pathway of signal transduction, SOCS, which inhibits the activation of transcriptional factor STAT-5 stimulated by other growth factors or cytokines.

Since both circulating IL-6 and IL-6R presumably play a key role in the pathogenesis of systemic-onset disease, and no medication except high-dose corticosteroids is available for the treatment of this disease, which will probably make the transition to the fatal condition macrophage activation syndrome, a new treatment approach is urgently needed, and it might be reasonable to select IL-6 and IL-6R as a candidate therapeutic target.

Development of MRA and its application

In the presence of IL-6, IL-6R has been shown to be able to associate with gp130 and to mediate IL-6 functions.[40] Thus, in order to block the biological functions of IL-6, three options could theoretically be available: a blockade of the gp130 receptor to abolish signal transduction, the neutralization of IL-6 itself, or the elimination of IL-6R to inhibit IL-6/IL-6R complex formation.

The IL-6/IL-6R complex induces gp130 homodimer formation, and the gp130 homodimer plays an important role in the formation of high-affinity IL-6 binding sites by associating with the IL-6/IL-6R complex in the transduction of the IL-6 signal. Moreover, gp130 is also utilized as a critical component in the IL-11 receptor complex,[41] and oncostatin M has been suggested to signal through a heterodimer receptor composed of oncostatin M-specific receptor component and gp130.[42] Since the functional redundancy of the IL-6 cytokine family might be explained by the homo- and heterodimer receptor of gp130, the blockade of gp130 means a wide-ranging shrinkage of IL-6 cytokine family functions.

In order to inhibit IL-6 function, a direct solution might be to eliminate circulating IL-6 molecules by monoclonal antibodies. Chimaeric anti-IL-6 monoclonal antibodies were given as B-cell growth factor to patients with multiple myeloma to inhibit IL-6 function, but none of the patients showed a response.[43] Anti-IL-6 monoclonal antibody therapy was given to patients with EB virus-induced posttransplant lymphoproliferative disorder. Although IL-6 has been described as a growth factor for EB virus-infected B cells, the efficacy of the therapy was limited.[44]

Anti-IL-6R antibody, MRA, is a genetically engineered monoclonal antibody that was humanized by the technique of complementary-determining region grafting from mouse antihuman IL-6R monoclonal antibody.[45] MRA has been shown to compete for both membrane–bound and soluble IL-6R, and to inhibit the formation of IL-6/IL-6R complex that results in the blockade of signal transduction through gp130. In animal experiments, MRA was shown to reduce joint inflammation and destruction in cynomolgus monkeys with collagen-induced arthritis.

Infusions of MRA in healthy adults and patients with rheumatoid arthritis were found to be well tolerated. For patients with rheumatoid arthritis, a randomized controlled trial was recently performed, and showed that the inhibition of IL-6 by intravenous infusion of MRA significantly improved the signs and symptoms of rheumatoid arthritis and

normalized the acute-phase reactants.[10] Clinical improvements appeared rapidly during the treatment period. The majority of adverse events were of mild intensity, and included diarrea or nausea.

Moreover, the successful inhibition of febrile episodes and inflammatory responses was achieved in patients with Castleman's disease,[11] and one patient with adult-onset Still's disease.[12] Infusions of 4 mg/kg MRA once every 2 weeks brought dramatic improvements in both clinical symptoms and laboratory abnormalities. A recent report indicated that Castleman's-disease-like symptoms in IL-6 transgenic mice were suppressed by MRA.[46]

MRA for children with systemic-onset JIA

The remarkable improvement after infusions of MRA of patients with rheumatoid arthritis, adult Still's disease, and Castleman's disease, in both clinical conditions and laboratory features, without severe adverse effects prompted us to conduct an MRA trial with children with systemic-onset JIA, which is also an IL-6-mediated disease.

Three children were treated with MRA for 14, 21, or 25 months. The clinical and laboratory responses to MRA infusions were prompt and remarkable. The spiking fever, rash, and intractable arthritis subsided within 4 days, and C-reactive protein levels decreased from over 10 mg/dl to below 1.0 mg/dl in 1 week. Scores in the disability domain of the Childhood Health Assessment Questionnaire began to improve at the first evaluation 2 weeks after the beginning of MRA treatment. The long-term effects of MRA were a remarkable catch-up growth (15–18 cm/year), and increased bone mineralization. Prednisolone was gradually tapered, and all oral medication was ceased at 12 weeks after the first infusion of MRA. No severe adverse events such as anaphylaxis or tuberculosis were observed during the course of the treatment.

The MRA therapy also gave a new dimension to the concept of systemic-onset JIA. Unlike other conventional therapies, MRA is a unique, single-point hit drug: the nature of MRA is a monoclonal antibody against IL-6R, and the target of this drug in vivo is quite simple, IL-6R molecules. The stabilization by MRA administration of clinical and laboratory features of systemic-onset disease, which seems to be complex because of the mixed characteristics of systemic inflammatory responses, indicates that IL-6 does play a central role in the pathogenic processes of this disease, and that the disease condition might progress as links to each manifestation, or as a cascade of events triggered by IL-6 and IL-6R imbalances. The improvement of growth and developmental impairment was achieved by MRA therapy, indicating that continuously elevated levels of serum IL-6 and consecutive signal transduction through gp130 results in growth failure in children with systemic-onset disease.

Conclusions

Children with systemic-onset JIA may suffer disease flares, adverse events from corticosteroids, and disease transition to macrophage activation syndrome. Recent advances in cytokine technology have given us a clearer understanding of the disease, and more new concepts for treatment strategies. Our experiences with MRA as a therapeutic option showed that the dramatic improvement in clinical symptoms and laboratory measures enabled us to withdraw prednisolone without flares, and strongly suggested that MRA could be a powerful therapeutic modality for children with systemic-onset JIA intractable to conventional therapy.

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