

# REVIEWS

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## Jak3 – Its Significance in Inflammatory Diseases of Joints – New Perspectives for Therapy

### Jak3 – znaczenie w zapalnych chorobach stawów – nowe perspektywy terapii

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#### Abstract

Jak3 is a cytoplasmic tyrosine kinase and its expression is limited to cells of immune system. This enzyme takes part in the signal transduction cascade of numerous growth factors, that are crucial for lymphocyte activations and play important roles in lymphocyte differentiation and proliferation. Based on its limited tissue distribution and the evidence of its role in immune cell functioning, Jak3 may represent an attractive target for immunosuppression and inhibitors of Jak3 seems to be important in the treatment of autoimmune diseases and in the prevention of transplant rejection (*Adv Clin Exp Med* 2008, 17, 4, 479–484).

**Key words:** arthritis, autoimmune diseases, Janus kinase, Jak/Stat system, inhibitors of Janus kinase.

#### Streszczenie

Jak3 jest wewnątrzcytoplazmatyczną kinazą tyrozynową, której ekspresja jest ograniczona do komórek układu immunologicznego. Enzym ten jest składnikiem układu przekazywania sygnału wielu czynników wzrostu, które mają decydujące znaczenie dla aktywacji limfocytów i odgrywają ważną rolę w różnicowaniu i proliferacji tych komórek. Ze względu na ograniczone występowanie w tkankach i znaczącą rolę w funkcjonowaniu komórek układu immunologicznego Jak3 może być atrakcyjnym celem immunosupresji, a inhibitory Jak3 wydają się istotne w leczeniu chorób autoimmunologicznych i w zapobieganiu odrzucania przeszczepów (*Adv Clin Exp Med* 2008, 17, 4, 479–484).

**Słowa kluczowe:** zapalenie stawów, choroby autoimmunologiczne, kinaza Janus, układ Jak/Stat, inhibitory kinazy Janus.

Inflammatory articular diseases of autoimmune nature, such as rheumatoid arthritis or seronegative spondyloarthropathies belong to diseases of a chronic and progressive course. Their natural development leads to destruction of joints and periarthicular structures as well as to multi-organ lesions. In pathogenesis of the diseases several cell types are involved, not only those representing immune system. The cells remain reciprocally interlinked by a network of cytokines, which most frequently act in multiple directions. Currently used classical drugs, which modify activity of rheumatoid disease (DMARDs), exert no selective effects and there-

fore also alter function of cells which are not involved in the inflammatory process and, may, induce several undesirable effects. Since activities of several cytokines and signal-transducing proteins continue to be appreciated, the need for new immune system-modulating drugs acting through control of production or activity of these molecules emerges. The perspective opens up for compounds, acting precisely on specific intracellular targets, that might induce the desired modulatory effect with significantly diminished undesirable effects. Blocking Jak3 tyrosine kinase seems to be one of these opportunities.

## Jak/Stat System

Jak3 tyrosine kinase belongs to the small group of intracellular enzymes, including Jak1, Jak2, Jak3, Tyk2. The kinase are activated by many different cytokines and growth factors with mediation of various receptors while Jak3 tyrosine kinase becomes activated, with mediation of the transmembrane receptor termed gamma chain ( $\gamma$ c), only by the following molecules: IL-2, IL-4, IL-7, IL-9, IL-15, IL-21 [1–3]. The cytokines bind also to other receptors but they stimulate Jak3 kinase exclusively through interaction with the gamma chain. Jak3 tyrosine kinase was noted to undergo a constitutive expression at a high level in NK cells (Natural Killer cells) and in thymocytes. Its expression can be induced in lymphocytes B, lymphocytes T and in bone marrow cells [4, 5]. Reports are also available on high expression of Jak3 kinase in synovial cells (including fibroblasts) in patients with rheumatoid arthritis or seronegative spondylo-arthropathies [6].

The enzyme constitutes a component of the system of Jak/Stat (Janus kinases/signal transducers and activators of transcription), the complex of proteins used by cytokines and growth factors for induction of gene expression, activation, proliferation and differentiation of cells.

Jak3 kinase is composed of two intracellular subunits, which become linked to each other following activation of the transmembrane receptor. This is followed by autophosphorylation of the enzyme and, thus, its activation. Subsequently, the active tyrosine kinase is joined by Stat subunits, through their SH2-pY domains, the subunits undergo phosphorylation and dimerization and, with mediation of transfer proteins, they become transported to the cell nucleus. In the cell nucleus the active Stat dimers bind to a specific promoter gene, and in this way activate the gene, inducing its expression. Phosphorylated Stat monomers were also found to be capable of activating gene expression [7, 8]. Currently seven Stat proteins are known, including Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, Stat6 and respective mouse phenotypes resulting from deletion of individual Stat proteins are presented in Table 1 [9].

Since Jak3 tyrosine kinase can be activated only by the gamma chain group of cytokines, binding of the receptor subunit to a specific activating molecule can be expected to yield specified effects. For example, IL-2 participates in activation of cytotoxic lymphocytes T and NK cells, in differentiation of Th cells to Th1 and Th2 cells and in control of tolerance to autologous antigens. An interesting activity is manifested by IL-4, thought to represent a factor able to inhibit development of an inflam-

**Table 1.** Stats, cytokines and phenotypes of Stat deficient mice

**Tabela 1.** Części Stat, cytokiny i poszczególne fenotypy myszy z utratą genu części Stat

STAT	Cytokines that activate (Cytokiny)	Phenotype of knockout (Fenotyp pozbawiony aktywności cytokiny)
STAT-1	type I IFNs, IFN- $\gamma$	impaired antiviral response; increased tumours; defective signalling in response to IFNs
STAT-2	type I IFNs	impaired antiviral response; defective signalling in response to IFNs
STAT-3	IL-10, GH, cytokines gp130 (LIF, IL-6, IL-11, OSM)	embryonically lethal; targeted disruption in macrophages and neutrophils showed defective IL-6 and IL-10 signalling; chronic enterocolitis
STAT-4	IL-12	defective Th1 differentiation
STAT-5a	cytokines $\beta$ chain ( $\beta$ c) (IL-3, IL-5, GM-CSF), PRL, GH, thrombopoietin	defective mammary gland development
STAT-5b	cytokines $\beta$ c, cytokines $\gamma$ chain ( $\gamma$ c), PRL, thrombopoietin	impaired sexually dimorphic growth
STAT-6	IL-4, -3	defective Th2 differentiation

LIF – Leucemia Inhibitory Factor.

GM-CSF – Granulocyte-Macrophage Colony-Stimulating Factor.

PRL – prolactin.

Cytokines  $\beta$ c –  $\beta$  chain cytokines.

LIF – czynnik hamujący białaczki

GM-CSF – czynnik wzrostu kolonii granulocytarno-makrofagowych

PRL – prolaktyna.

Cytokines  $\beta$ c – cytokiny łańcucha  $\beta$ c.

matory process. The cytokine stimulates differentiation of Th2 cells, slows down differentiation of Th1 cells and macrophage activation. It controls also function of lymphocytes B and, in cooperation with IL-21, controls switching of immunoglobulin classes [10, 11]. Function of individual gamma chain cytokines is presented in Table 2.

## Jak3 and SCID

In mice, loss of function of individual tyrosine kinases results in severe damage to the organism and frequently is lethal. Deletion of Jak1 kinase

**Table 2.** Cytokines which share the  $\gamma c$  receptor and their functions**Tabela 2.** Cytokiny, które łączą się z łańcuchem gamma i ich funkcje

Cytokine (Cytokina)	Functions (Funkcje)
IL-2	clonal expansion of T cells; activation of cytotoxic T lymphocytes and NK cells; differentiation of T helper (Th) cells; regulation of self-tolerance and development of regulatory T cells (T regs)
IL-4	enhancement of T helper 2 (Th2) cell differentiation; antagonism of Th 1 differentiation and macrophage activation; regulation of B cell function and immunoglobulin class switching in concert with IL-21; stimulation of mast cells
IL-7	development of T and B cells; homeostasis of peripheral lymphocytes; generation of CD8+ memory T cells
IL-9	goblet cell hyperplasia and mucus production
IL-15	development, differentiation, survival, and activation of NK cells; homeostasis of peripheral T cells; generation of CD8+ memory T cells
IL-21	regulation of B cell function and immunoglobulin class switching in conjunction with IL-4; proliferation and activation of NK cells

CD – cluster of differentiation.

CD – kompleks różnicowania.

results in a severe neurological injuries, in severe combined immunodeficiency (SCID) and perinatal mortality. Loss of Jak2 kinase function leads to abnormal erythropoiesis and foetal mortality. Deletion of the gene for Tyk2 kinase results in a decreased sensitivity to viral infections and in resistance to induction of arthritis. Therefore, it would be worth while to consider chances for taking advantage of modulating activity of the enzyme in therapy of inflammatory diseases of joints. On the other hand, deletion of Jak3 tyrosine kinase is not lethal but results in the severe complex immunodeficiency (SCID). Therefore, extensive hopes are linked to modulation of the enzyme activity in treatment of autoimmune diseases. The syndrome manifests itself by the T-B+NK-phenotype, i.e., no T and NK cells are present, and it is inherited in the autosomal dominant manner. SCID represents a syndrome of severe inborn defects in immunity, with frequent incidence of severe, frequently opportunistic infections and it leads to death in the first years of life. Such disturbances can be effectively cured by bone marrow transplantation.

However, SCID may result not only from deletion of Jak3 kinase gene. A SCID phenotype similar to that resulting from deletion of Jak3 (T-B+NK-) gene, but inherited in the manner linked to X chromosome, may reflect from deletion of the gene coding for the transmembrane gamma  $\gamma c$  receptor. Although in the latter case the block involves signalling mediated by IL-4, IL-7, IL-15 and IL-21 only, effects of the receptor absence are equally severe: in either case the lack of T cell regulatory function leads to impoverished activation of B cells and a disturbed antibody production. Clinically, this is manifested by hypogammaglobulinaemia and a disturbed production of antibodies in response to immunisation [12, 13].

In humans, approximately 10% of SCID cases are noted to reflect deletion of the receptor for IL-7, linked to Jak1 tyrosine kinase. However, in this case the manifested phenotype differs from the earlier described ones: B and NK cells are present but T cells (T-B+NK+) and their regulatory function are absent. This form of SCID is inherited in the autosomal dominant manner. Effects of deletion involving individual kinases in mice are presented in Table 3 [8].

## Inhibitors of Jak/Stat system

Considering the observations on SCID pathogenesis, blockade of Jak3 kinase function may provide a promising element in therapy of diseases of autoimmune background, including seronegative spondyloarthropathies and rheumatoid arthritis. Blocking of Jak3 activity may be thought to alleviate the inflammatory response and signs/symptoms of the diseases. This might be obtained by application of designed inhibitors of Jak3 or by activation of natural inhibitors targeting the Jak/Stat system. The latter group of proteins includes SOCS (suppressors of cytokine signalling-SOCS) proteins, PIAS (protein inhibitors of activated Stats) and tyrosine phosphatases (PTP), which inhibit at various levels activity of Jak/Stat system. SOCS proteins represent classical reciprocal inhibitors of signalling. Their expression takes place following activation of Jak/Stat system. They prevent the signalling process by inhibiting Jak activity, by preventing recruitment of signalling molecules to a cytokine receptor and by promotion of cytokine receptor and Jak kinase degradation. The mentioned above PIAS proteins represent inhibitors of Stat activation. The molecules bind to Stat monomers blocking their dimerization and phosphorylation and, in this way, blocking activation and expression of specific genes. On the other

**Table 3.** The Janus kinase family and cytokines reported to activate them**Tabela 3.** Rodzina kinaz Janus i cytokiny, które je aktywują

Jak	Cytokines that activate (Cytokiny)	Phenotype of knockout (Fenotyp pozbawiony aktywności enzymu)
Jak1	gp130cytokines (IL-6, IL-11, IL-12, OSM, CNTF, LIF, Leptin, CT-1), IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , $\gamma$ c cytokines	perinatally lethal; neurologic defects; SCID
Jak2	EPO, thrombopoetin, PRL, GH, $\beta$ c cytokines, IFN- $\gamma$ , IL-12	embryonically lethal; defective erythropoiesis
Jak3	$\gamma$ c cytokines	AR-SCID
Tyk2	gp130 cytokines, IFN- $\alpha$ , IFN- $\beta$ , IL-12, IL-23	modest viral susceptibility; reduced IL-12 response and resistance to arthritis

TNF – Tumor Necrosis Factor.

IL – interleukin.

IFN – interferon.

GH – Growth Factor.

OSM – Oncostatin M.

CNTF – Ciliary Neurotrophic Factor.

CT-1 – Cardiotrophin 1.

EPO – erythropoietin.

TNF – czynnik martwicy nowotworu.

IL – interleukina.

IFN – interferon.

GH – czynnik wzrostu.

OSM – onkostatyna.

CNTF – rzęskowy czynnik neurotropowy.

CT-1 – kardiotropina 1.

EPO – erytropoetyna.

hand, tyrosine phosphatases diminish cytokine signaling by dephosphorylation of cytokine receptors, Jak tyrosine kinases and Stat molecules. Activators of tyrosine phosphatase, molecules of PIAS function or molecules mimicking function of SOCS might carry the potential of novel immunosuppressive drugs [14, 15].

## Dendritic Cells and Jak3 Inhibitors

Dendritic cells (DCs) are of key importance for initiation of inflammatory reaction. They belong to the group of cells which present antigen. Moreover, they stimulate cell-mediated and humoral immune responses, promote function of non-specific immunity cells, secure immune tolerance and, maturing, they increase their numbers of surface MHC I (Major Histocompatibility Antigen), MHC II, CD40, CD54, CD80, CD86

molecules and intensity of IL-4, IL-6, IL-12, IFN- $\gamma$  production. Two types of dendritic cells can be distinguished, including myeloid dendritic cells (moPDCs) and plasmacytoid dendritic cells (PDCs). Subpopulations of the cells are differentiated on the basis of CD11c and CD123 expression: moDCs carry CD11c- CD123+ phenotype and morphology of monocytoid cells. The cells may differentiate to CD14+ monocytoid derivative blood cells, to Langerhans cells in the skin or in intestines or, in other words, to typical antigen-presenting cells. Following activation, moDCs mature and migrate to secondary lymphoid organs, in which they initiate immune response. Mature moDCs produce high amounts of IL-12 and IFN- $\gamma$ , which play a key role in initiation of inflammatory type Th1 responses and in release of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 [16–20]. The cytokines are known to be of basic importance in pathogenesis of joint inflammatory diseases. Plasmacytoid dendritic cells are distinguished by their CD11c-, CD123+ (a IL-3R chain), CD4+, CD45RA+, BDCA-2, BDCA-4 (Blood Dendritic Cells Antigen) phenotype and by their morphology resembling that of plasma cells [21]. They exhibit less pronounced antigen presenting potential than that noted in moDCs, possibly due to the absence of cathepsins S and D, low expression of co-stimulatory molecules and their low level of MHC II antigen expression. The cells produce IFN- $\alpha$ , IFN- $\beta$ , IL-6, which participate in generation of plasma cells, in production of antibodies as well as in initiation and intensification of T cell activation in the course of viral infection. Phenotypic differences have been described between synovial dendritic cells in patients with rheumatoid arthritis and dendritic cells of healthy synovium [22]. Moreover, results of a few studies in patients with seronegative spondyloarthritis or rheumatoid arthritis demonstrated numerical prevalence of dendritic cells in synovium and in synovial fluid over other cells of inflammatory process [23, 24]. On the other hand, Walker and collaborators examined expression of Jak/Stat in synovial cells of patients with rheumatoid arthritis and noted that just the immature (CD1a) dendritic cells manifested the most pronounced expression of Jak3 and STAT-4. Few mature DC were noted with expression of Jak3 and STAT-4 (DCs CD83 p55) and no expression of Jak3 and STAT-4 was observed in CD45 cells (lymphocytes T), CD22 cells (lymphocytes B), CD68 cells (macrophages) or CD 55 cells. Moreover, the cells which produced significant amounts of IL-12, IFN- $\alpha$  and IFN- $\gamma$  were found to involve cells with highly pronounced expression of Jak3 and STAT or dendritic cells at a variable grade of maturity [22].

In view of the above it may seem that just dendritic cells might provide the target for inhibitors of Jak3 tyrosine kinase. A few other compounds with such properties have been described but most of them inhibits also activity of the remaining Jak kinases and, therefore, they result in exceedingly numerous undesirable effects to link with them hopes for therapeutic application. The compound termed CP-690550 belongs to substances which block Jak3 kinase to a much more pronounced extent than it affects the other kinases.

Its *in vitro* efficacy against Jak3 kinase is noted in nanomolar concentrations (enzyme inhibitory potency of 1nM). Moreover and very importantly, as compared to Jak3, it exerts a 30-fold lower effect on Jak2 and a 100-fold lower effect on Jak1. But it was found to induce no granulocytopenia and to induce mild anaemia only, despite effective blocking of Jak2. Because mediates signaling via many hematopoietic cytokines (erythropoietin, trombopoietin) potent Jak2 inhibition could result anemia or thrombocytopenia. In animal graft models, the agent significantly prolonged survival of heart allografts and proved to be the immunosuppressant more effective than

cyclosporinA in preventing kidney graft rejection in animals. CP690550 results in an insignificant decrease in numbers of lymphocytes T, CD8+ cells and NK cells [25]. The promising results of the observations found their continuation in studies using CP-690550 in two groups of rats with the induced rheumatoid arthritis: adjuvant induced arthritis (AA) or collagen induced arthritis (CIA). In both groups recession of clinical signs of arthritis and alleviation of histopathological lesions in joints was observed following application of CP-690550 inhibitor of Jak3. Moreover, the agent demonstrated clinical efficacy higher than that of anti-TNF- $\alpha$  and decreased serum IL-6 level to the extent similar to effects of anti-TNF- $\alpha$  [26].

The authors concluded that observations of patients with mutation of Jak3 gene or gamma chain-coding gene demonstrate that selective inhibitors of Jak3 may in future provide a new class in immunosuppressive drugs. Data on CP-690550 seem to confirm the perspective. The latter agent is effective in treatment on animal models of arthritis, they reduce both clinical and histopathological manifestations of the disease, including lesions to articular cartilage and to bone tissue.

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