AOP ID and Title:

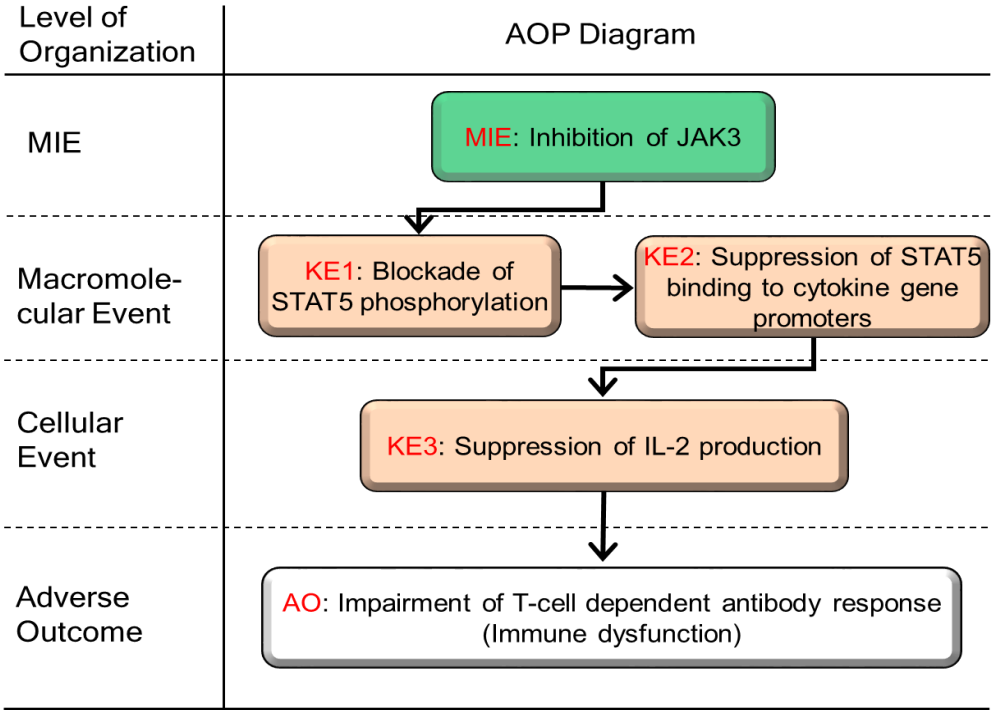
SNAPSHOT

Created at: 2020-05-18 09:23

AOP 315: Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response

Short Title: Immune dysfunction induced by JAK3 inhibition

Graphical Representation



Authors

Yasuhiro Yoshida (1) Takao Ashikaga (1) Tomoki Fukuyama (1) Ken Goto (1) Shinko Hata (1) Shigeru Hisada (1) Shiho Ito (1) Hiroyuki Komatsu (1) Sumie Konishi (1) Tadashi Kosaka (1) Kiyoshi Kushima (1) Shogo Matsumura (1) Takumi Ohishi (1) Yasuharu Otsubo (1) Junichiro Sugimoto (1)

(1) AOP Working Group, Testing Methodology Committee, The Japanese Society of Immunotoxicology

Corresponding author: Yasuhiro Yoshida (freude@med.uoeh-u.ac.jp)

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.74	Included in OECD Work Plan

Abstract

Signal transduction between immune-related cells depends in many cases on cytokines and takes place via cell surface cytokine receptors as well as direct cell-to-cell interaction. Cytokines influence the movement, proliferation, differentiation, and activation of lymphocytes and other leukocytes in a variety of ways.

Some receptors for cytokines require and activation step through a Janus-kinase (JAK) Signal Transducers and Activator of Transcription (STAT) system. When cytokine binds to its specific cytokine receptors, the cytokine receptors form dimers, which more closely resemble the JAK molecules. The JAK then activates to phosphorylate adjacent cytokine receptors. STATs bind to the phosphorylated sites of the receptors and

are then phosphorylated by the activated JAK. The phosphorylated STAT is dimerized to be translocated into nucleus and bind to promoter regions of cytokine genes, which starts transcription of cytokine genes in the nucleus.

In mammals, four JAK families of enzymes (JAK1, JAK2, JAK3, TYK2) and seven STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6) are utilized by more than 50 cytokines and growth factors to mediate intracellular signaling. In particular, pro-inflammatory cytokines such as interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-4, IL-6, IL-13, IL-21 and IL-23 have been implicated in inflammatory diseases that utilize the JAK pathway. In addition, TH2 derived cytokines, including IL-31 and thymic stromal lymphopoietin (TSLP), are ligands for murine and human sensory nerves and have a critical function that evokes itchiness. Because these cytokines also interact with JAK, several JAK-inhibitors have received a lot of attention recently as a therapeutic agent for major inflammatory diseases and pruritic diseases. However, immunotoxic events due to inhibition of the JAK pathway have yet to be examined.

This AOP focuses on the inhibition of JAK3, which is required for signal transduction by cytokines through the common gamma (γ) chain of the interleukin receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. This AOP proposes JAK3 inhibition as an MIE that leads to suppression of T cell-dependent antibody response (TDAR) as an AO. TDAR is frequently affected under immunosuppressive conditions and is a major endpoint in many preclinical immunotoxicity studies. In this proposed AOP, JAK3 selective inhibitors (e.g. PF-06651600, RB1) are stressors, blockade of STAT5 phosphorylation is KE1, suppression of STAT5 binding to the promoter regions of cytokine genes is KE2, and subsequent suppression of IL-2 production is KE3.

Background

Although there are numerous stressors that inhibit JAK3 activity, this AOP is based on immunosuppression caused by recently developed, highly selective JAK3 inhibitors PF-06651600 and RB1, about which a significant body of scientific literature has been published.

We look forward to future amendments to this AOP with up-to-date information on other stressors, which will clarify the link between inhibition of JAK activity and impairment of TDAR.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1715	Inhibition of JAK3 (https://aopwiki.org/events/1715)	Inhibition of JAK3
	KE	1716	Blockade of STAT5 phosphorylation (https://aopwiki.org/events/1716)	STAT5 inhibition
	KE	1717	Suppression of STAT5 binding to cytokine gene promoters (https://aopwiki.org/events/1717)	Suppression of STAT5 binding
	KE	1718	Suppression of IL-2 production (https://aopwiki.org/events/1718)	Suppression of IL-2 production
	AO	1719	Impairment of T-cell dependent antibody response (https://aopwiki.org/events/1719)	Impairment, TDAR

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Inhibition of JAK3 (https://aopwiki.org/relationships/2024)	adjacent	Blockade of STAT5 phosphorylation	High	High
Blockade of STAT5 phosphorylation (https://aopwiki.org/relationships/2025)	adjacent	Suppression of STAT5 binding to cytokine gene promoters	High	High

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Suppression of STAT5 binding to cytokine gene promoters (https://aopwiki.org/relationships/2026)	adjacent	Suppression of IL-2 production	High	High
Suppression of IL-2 production (https://aopwiki.org/relationships/2027)	adjacent	Impairment of T-cell dependent antibody response	High	High

Overall Assessment of the AOP

Janus kinases (JAKs) are a family of nonreceptor tyrosine kinase and consists of four members: JAK1, JAK2, JAK3, and Tyk2 (1). All four members mediate signals initiated by cytokines through interactions with receptors for IL-2, IL-5, IL-7, IL-9, and IL-15 via the common γ chain (2). Previous studies with IL-2Rγ-null mice showed that JAK3 is related to the development of spontaneous IBD symptoms (3). Moreover, abnormal activation of JAK3 was associated with human hematological (4), indicating that a tight balance of its activity was essential for normal hematopoietic development.Janus kinases (JAKs) are a family of nonreceptor tyrosine kinase and consists of four members: JAK1, JAK2, JAK3, and Tyk2 (1). Different studies have shown that JAK3 is widely expressed in different organs (2). Previous studies with IL-2Rγ-null mice showed that JAK3 is related to the development of spontaneous IBD symptoms (3). Moreover, abnormal activation of JAK3 was associated with human hematological (4), indicating that a tight balance of its activity was essential for normal hematopoietic development.

Although JAK1, JAK2, and Tyk2 are each widely expressed, JAK3 is predominantly expressed in hematopoietic cells and is known to associate only with the common γ (γc) chain of the interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15 receptors (5). Homozygous mutant mice in which the JAK3 gene had been disrupted were generated by gene targeting. JAK3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to severe combined immunodeficiency disease (SCID), and the residual T cells and B cells were functionally deficient. Thus, JAK3 plays a critical role in γc signaling and lymphoid development.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Sex Applicability

Sex	Evidence
Mixed	High

This proposed AOP involves inhibition of JAK activity leading to suppression of TDAR and is not dependent on life stage, sex, or age. Since JAK3 inhibitors (PF-06651600, RB1) are currently under a phase 2 clinical evaluation to treat rheumatoid arthritis, the AOP appears to be applicable to all life stages. Since JAK3 inhibitor-induced outcomes in humans are mimicked by similar responses in a variety of animal models, including non-human primates and rodents, immunosuppression induced by inhibition of JAK3 activity is considered to occur across a variety of mammalian species.

Essentiality of the Key Events

MIE and later events: JAK3-knockout (KO) mice

JAK3 was initially identified (1,2) in studies to identify the JAK family member that was involved in the signaling of a group of cytokines that shared in common the utilization of the γc chain first identified in the interleukin 2 (IL-2) receptor complex. It was subsequently demonstrated that JAK3 physically associates with the γc chain and is activated in a receptor complex that also contains JAK1, which associates with the ligand specific alpha or beta chain of the receptors (3). JAK3 is somewhat unique within the JAK family in that it is predominantly expressed in

hematopoietic cells and is only activated in the responses to cytokines that use the γc chain (4). The phenotype of the JAK3 deletion mice was quite striking and consisted of a range of deficiencies which collectively constituted SCID (5,6). At the same time, two groups identified individuals that lacked JAK3 and exhibited somatically acquired SCID (7,8). One of the most striking components of the phenotype is the dramatic reduction seen in both the T and B cell lineages. Comparable reductions are seen in mice that lack IL-7 (9), the IL-7 receptor alpha chain (10), or the γc chain. In spite of the reduced numbers, the cells that do develop are phenotypically normal. These results are consistent with the hypothesis that activation of JAK3 give it a critical role in the expansion but not the differentiation of early lymphoid lineage-committed cells. In addition to the reduced numbers, the differentiated lymphoid cells that are generated fail to respond to the spectrum of cytokines that utilize the γc chain and activate JAK3 normally.

Stressor: B6.Cg-Nr1d1tm1Ven/LazJ mouse

The B6.Cg-Nr1d1tm1Ven/LazJ mouse line harbors a spontaneous mutation in JAK3, which generates an SCID phenotype with an inability to generate antigen-independent professional cytokine-producing innate lymphoid cells (ILCs). Mechanistically, JAK3 deficiency blocks ILC differentiation in the bone marrow at the ILC progenitor (ILCP) and the pre-NK cell progenitor (pre-NKP). Similar phenomenon was further demonstrated by the pan-JAK inhibitor tofacitinib and specific JAK3 inhibitor PF-06651600. Both JAK-inhibitors impair the ability of human intraepithelial ILC1 (iILC1) to produce IFN-γ, without affecting ILC3 production of IL-22. Both inhibitors impaired the proliferation of iILC1 and ILC3 and differentiation of human ILC in vitro. These findings indicate that JAK3 deficiency blocks innate lymphoid cell development (11).

KE1 and later events: STAT5-KO mice

STAT5 plays a major role in regulating vital cellular functions such as proliferation, differentiation, and apoptosis of hematopoietic and immune cells (12,13). STAT5 is activated by phosphorylation of a single tyrosine residue (Y694 in STAT5) and negatively regulated by dephosphorylation. A wide variety of growth factors and cytokines can activate STAT5 through the JAK-STAT pathway. The activation of STAT5 is transient and tightly regulated in normal cells (14).

The following phenotypes are observed in STAT5-KO mice:

The transcription factor STAT5 is expressed in all lymphocytes and plays a key role in multiple aspects of lymphocyte development and function (15). STAT5 was initially identified as a transcription factor activated by prolactin in mammary gland epithelial cells (16,17). Subsequent studies identified STAT5 binding activity in T cells (18), and it was later established that STAT5 was expressed in multiple cell types and activated by a number of cytokines, including the common gamma chain (γc)-dependent cytokines interleukin 2 (IL2), IL4, IL7, IL13, and IL15 (19).

STAT5 in T-cell development

The observation that STAT5 is activated by multiple cytokines in T cells suggested that it might play a critical role in the development or function (or both) of these cells. Disruption of Stat5a or Stat5b genes alone resulted in relatively modest phenotypes; for example, Stat5a-/- mice had defects in mammary gland development and lactation while Stat5b-/- mice had defects in response to growth hormone in male mice and natural killer cell proliferation (20,21). To determine whether combined deletion of Stat5a and Stat5b might result in more profound immunodeficiencies, subsequent studies deleted the first coding exons of both Stat5a and Stat5b. This intervention resulted in the production of truncated forms of STAT5a and STAT5b that acted as functional hypomorphs. These mice too had surprisingly mild defects in lymphocyte development, although T cells were grossly dysfunctional, as they could no longer proliferate in response to IL2 (22,23). Finally, complete deletion of Stat5a and Stat5b using Cre-LoxP approaches demonstrated that STAT5a and STAT5b are absolutely required for lymphocyte development, as Stat5a/b-/- mice had profound blocks in lymphocyte development, which mimicked that observed in Il7r-/- mice (24,25). These studies definitively demonstrated that the STAT5 hypomorph mice retained significant STAT5 function.

Weight of Evidence Summary

Biological Plausibility

T-cell development is mainly regulated by JAK-STAT system, and JAK3 deficiency in T cells is known to induce multiple types of immunosuppression, including T cell-dependent antibody response (TDAR).

JAK3-deficient mice had profound reductions in thymocytes and severe B cell and T cell lymphopenia similar to SCID disease, and the residual T cells and B cells were functionally deficient (10).

Mice lacking JAK3 also showed a severe block in B cell development at the pre-B stage in the bone marrow. In contrast, although the thymuses of these mice were small, T cell maturation progressed relatively normally. In response to mitogenic signals, peripheral T cells in JAK3-deficient mice did not proliferate and secreted small amounts of IL-2. These data demonstrate that JAK3 is critical for the progression of B cell development in the bone marrow and for the functional competence of mature T cells (5).

Furthermore, the abnormal architecture of lymphoid organs suggested the involvement of JAK3 in the function of epithelial cells. T cells developed in the mutant mice did not respond to either IL-2, IL-4, or IL-7 (26).

Specific JAK3 inhibitor PF-06651600 or RB1, which selectively inhibited JAK3 with an over 100-fold preference over JAK2, JAK1, and TYK2 in the kinase assay, displayed reduced inflammation and associated pathology in collagen-induced-arthritis mice. Importantly, with PF-06651600 or RB1 administration, pro-inflammatory cytokines and JAK3 and STATs phosphorylation decreased in mice, suggesting that the inhibition of JAK3/STAT signaling was closely correlated with induction of multiple types of immunosuppression, including TDAR .

Quantitative Consideration

KER1 (MIE=>KE 1)

Interleukin 2 (IL-2) activated STAT5 via distinct pathways (30).

IL-2 have been demonstrated to stimulate STAT5 and induce tyrosine phosphorylation of STAT5. Treatment of highly selective JAK3 inhibitors

(PF-06651600 or RB1) treatment clearly suppresses the complex formation of STAT5 in the nucleus.

Highly-selective JAK3 inhibitor RB1 inhibited the phosphorylation of STAT5 elicited by IL-2 at IC50 value of 31 nM in the raw peripheral blood mononuclear cells (PBMCs) of humans. PBMCs were isolated from the buffy coats of healthy volunteers using density gradient centrifugation on Lymphoprep. Cells were cultured in complete RPMI 1640 medium (containing 10% foetal bovine serum, 100 mg/ml streptomycin and 100 U/ml penicillin) plus 10 µg/ml lectin phytohemagglutinin (PHA) for 3 days and then treated with either recombinant human IL-6 (400 ng/ml), recombinant human IL-2 (100 ng/ml), or recombinant human GM-CSF (50 ng/ml) at 37 °C for 20 min. To terminate the stimulation, cells were fixed with Lyse/Fix Buffer and then incubated with 100% methanol for 30 minutes; cells were incubated with anti-pSTAT3 and anti-CD4 Abs, or anti-pSTAT5 and anti-CD4 Abs at 4 °C overnight, washed twice with PBS, and analysed with an flow cytometer (31).

Fluorescence intensity for phospho-STAT5 in CD3-positive lymphocytes increased upon incubation of peripheral blood with IL-2. Peficitinib inhibited STAT5 phosphorylation in a concentration-dependent manner with a mean IC50 of 124 nM (101 and 147 nM for two rats). Additionally, the effect of peficitinib on IL-2 stimulated STAT5 phosphorylation in human peripheral T-cells was evaluated. Paralleling results in rats, the fluorescence intensity of phospho-STAT5 in CD3-positive lymphocytes increased in human peripheral blood after adding IL-2, but peficitinib inhibited STAT5 phosphorylation in a concentration-dependent manner with a mean IC50 of 127 nM in human lymphocytes (26).

KER2 (KE1 =>KE 2)

IL-2 activated STAT5 (30).

IL-2 have been demonstrated to stimulate STAT5 and induce tyrosine phosphorylation of STAT5. These IL-2-induced STATs have an identical DNA binding specificity and immunoreactivity.

KER3 (KE2 =>KE 3)

IL-2 activated STAT5 (30)

IL-2 have been demonstrated to stimulate STAT5 and induce tyrosine phosphorylation of STAT5. These IL-2-induced STATs have an identical DNA binding specificity and immunoreactivity.

Gel mobility shift assay showed that IL-2 activation induced STAT5 dimerization and DNA binding to gamma interferon-activated site (GAS) motif in IL-2 promoter region (32).

KER4 (KE3 =>AO)

As for IL-2 and antibody production, in vitro T-cell-induced polyclonal B cell activation to produce antibody was inhibited with anti-IL-2 and anti-IL-2R antibodies (33). In addition, cynomolgus monkeys treated with CsA showed suppression of IL-2 and TDAR using sheep red blood cells with a dose dependent manner (34).

In the human T-B cell co-culture stimulated with anti-CD3 monoclonal antibody, CNIs of FK506 and CsA lowered the levels of T-cell cytokines including IL-2 and IL-4 and inhibited IgM and IgG productions with a dose-dependent manner (35).

These results show the quantitative relationships between the inhibition of IL-2 by specific antibodies or CNI and suppression of antibody production.

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Appendix 1

List of MIEs in this AOP

Event: 1715: Inhibition of JAK3 (https://aopwiki.org/events/1715)

Short Name: Inhibition of JAK3

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:315 - Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	MolecularInitiatingEvent

Biological Context

Level of Biological Organization
Molecular

List of Key Events in the AOP

Event: 1716: Blockade of STAT5 phosphorylation (https://aopwiki.org/events/1716)

Short Name: STAT5 inhibition

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:315 - Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Event: 1717: Suppression of STAT5 binding to cytokine gene promoters (https://aopwiki.org/events/1717)

Short Name: Suppression of STAT5 binding

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:315 - Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Event: 1718: Suppression of IL-2 production (https://aopwiki.org/events/1718)

Short Name: Suppression of IL-2 production

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:315 - Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	KeyEvent

Biological Context

Level of Biological Organization
Cellular

List of Adverse Outcomes in this AOP

Event: 1719: Impairment of T-cell dependent antibody response (https://aopwiki.org/events/1719)

Short Name: Impairment, TDAR

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:315 - Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	AdverseOutcome

Biological Context

Level of Biological Organization
Individual

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2024: Inhibition of JAK3 leads to STAT5 inhibition (https://aopwiki.org/relationships/2024)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	**adjacent**	**High**	**High**

Relationship: 2025: STAT5 inhibition leads to Suppression of STAT5 binding (https://aopwiki.org/relationships/2025)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	**adjacent**	**High**	**High**

Relationship: 2026: Suppression of STAT5 binding leads to Suppression of IL-2 production

(https://aopwiki.org/relationships/2026)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	**adjacent**	**High**	**High**

Relationship: 2027: Suppression of IL-2 production leads to Impairment, TDAR (https://aopwiki.org/relationships/2027)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of JAK3 leading to impairment of T-Cell Dependent Antibody Response (https://aopwiki.org/aops/315)	**adjacent**	**High**	**High**