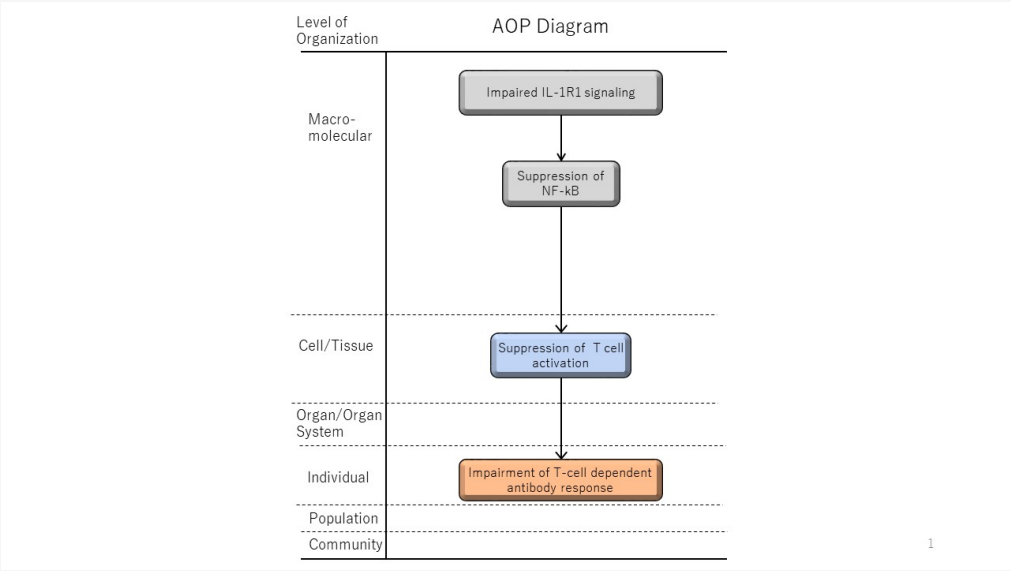
## AOP ID and Title:

AOP 277: Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response
**Short Title: IL-1 inhibition**

## Graphical Representation




## Authors

Yutaka Kimura (1) Setsuya Aiba (1) Takao ashikaga (2) Takumi Ohishi (2) Kiyoshi Kushima (2)

(1) Depertment of Dermatology, Tohoku University Graduate School of Medicine

(2) The Japanese Society of Immunotoxiclology

Corresponding author: Takao Ashikaga

## Status

| Author status | OECD status | OECD project | SAAOP status |
|---|---|---|---|
| Open for citation & comment | EAGMST Under Review | 1.48 | Included in OECD Work Plan |

## Abstract

The pleiotropic cytokine IL-1 mediates its biological functions via association with the signaling receptor IL-1R1. These may include initiation of innate immunity as well as acquired immunity, which are essential for assistance of host defense against infection. The trimeric complex consists of IL-1, IL-1R1 and IL-1R3 (a coreceptor, formerly IL-1R accessory protein) allows for the approximation of the Toll-IL-1-Receptor (TIR) domains of each receptor chain. MyD88 then binds to the TIR domains. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB. In addition to the NF-κB pathway, IRAK activates a variety of transcription factors, including Adaptor protein-1 (AP-1). The activation of NF-κB plays a principal role in the immunological function of IL-1. Namely, it stimulates innate immunity such as activation of dendritic cells and macrophages. It also stimulates T cells via activated dendritic function or directly. The activation of T cells is crucial for B cell proliferation and their antibody production. The cooperation by T cells and B cells constitutes a main part of host defense against infection. Therefore, the impaired IL-1R1 signaling either by the decreased IL-1 production or the inhibition of IL-1β binding to IL-1R1 by IL-1 receptor antagonist IL-1Ra or anti-IL-1β antibody) results in the blockade of the effects of the pleiotropic cytokine IL-1β leading to T cell dependent antibody response (TDAR).

In this AOP, we selected the impaired IL-1R signaling as a molecular initiating event (MIE) in T cell, and suppression of NF-κB (and/or AP-1), suppression of T cell activation, and suppression of TDAR as key events (KE).

Although the purpose of this AOP is to elucidate biological pathways that lead to increased immune suppression caused by impaired IL-1R signaling by chemicals, most of the stressors presented in this AOP were limited to pharmaceuticals because of the lack of information on chemicals.

## Background

The pleiotropic cytokine IL-1 mediates its biological functions via association with the signaling receptor IL-1R1. These may include initiation of innate immunity and assistance of host defense, and sometimes, mediation of autoinflammatory, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever. The trimeric complex consists of IL-1, IL-1R1 and IL-1R3 (a coreceptor, formerly IL-1R accessory protein) allows for the approximation of the Toll-IL-1-Receptor (TIR) domains of each receptor chain. MyD88 then binds to the TIR domains. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB and/or AP-1 and fundamental inflammatory responses such as the induction of cyclooxygenase type 2, production of multiple cytokines and chemokines, increased expression of adhesion molecules, or synthesis of nitric oxide. (Dinarello, 2018; Weber et al., 2010a, b; Jain et al., 2014).

Molecules like nuclear or mitochondrial DNA, adenosine triphosphate (ATP), uridine triphosphate (UTP), uric acid and high mobility group box 1 (HMGB1) are classified as damage associated molecular patterns (DAMPs). DAMPs are secreted or produced upon cellular injury or death and induce sterile inflammation. On the other hand, bacterial products like lipopolysaccharide (LPS), peptidoglycans, lipoprotein flagellins, bacterial RNA and DNA are some of the well-characterized pathogen associated molecular patterns (PAMPs). These DAMPs and PAMPs with a few exceptions bind to pattern recognition receptors (PRRs) such as toll-like receptor (TLRs) and nucleotide oligomerization domain (NOD) like receptors (NLRs). Proinflammatory mediators such as DAMPs, PAMPs, and various inflammatory cytokines or mediators including IL-1β itself activate innate immune mechanisms in the host leading to IL-1β production (Handa et al., 2016; Newton and Dixit, 2012; Yang et al., 2017). Besides transcriptional regulation and posttranscriptional level by RNA-binding proteins, pro-IL-1β protein requires proteolytic cleavage by active caspase-1 as the effector component of stimulation-induced multi-protein inflammasomes to acquire functional activity. Altogether, these different layers of regulation allow to fine tune IL-1β production under different pathophysiological conditions (Bent et al., 2018).

Therefore, the inhibition of various targets in different layers from the stimulation of PRRs or the receptors of proinflammatory cytokines, e.g., IL-1, IL-18, or TNFa, to the activation of NF-κB and/or AP-1 or the inhibition of posttranscriptional regulation of pro-IL-1β cause impaired IL-1R1 signaling. In addition, since IL-1 also mediates autoinflammatory syndromes, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever, several inhibitors against IL-1R1 have been developed. They are IL-1 receptor antagonist IL-1Ra , anakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R). Several reports described that the administration of these drugs led to increased susceptibility to infection(De Benedetti et al., 2018; Fleischmann et al., 2003; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Migkos et al., 2015; Schlesinger et al., 2012; Yokota et al., 2017). In addition to these human data, the experiments using knockout mice revealed that the lack of IL-1 signaling led to bacterial, tuberculosis or viral infection(Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Tian et al., 2017; Yamada et al., 2000).

## Summary of the AOP

### Events

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

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| 1 | MIE | 1700 | Impaired IL-1R1 signaling in T cell | Impaired IL-1R1 signaling |
| 2 | KE | 202 | Inhibition, Nuclear factor kappa B (NF-kB) | Inhibition, Nuclear factor kappa B (NF-kB) |
| 3 | KE | 1702 | Suppression of T cell activation | Suppression of T cell activation |
| 4 | AO | 986 | Impairment of TDAR | Impairment, Impairment of TDAR |

### Key Event Relationships

| Upstream Event | Relationship Type | Downstream Event | Evidence | Quantitative Understanding |
|---|---|---|---|---|
| Impaired IL-1R1 signaling in T cell | adjacent | Inhibition, Nuclear factor kappa B (NF-kB) | High | Moderate |
| Inhibition, Nuclear factor kappa B (NF-kB) | adjacent | Suppression of T cell activation | High | Moderate |
| Suppression of T cell activation | adjacent | Impairment of TDAR | High | High |

### Stressors

| Name | Evidence |
|---|---|
| IL-1 receptor antagonist IL-1Ra (Anakinra) | High |
| anti-IL-1b antibody (Canakinumab) | High |
| soluble IL-1R (Rilonacept) | High |
| anti-IL-1b antibody (Gevokizumab) | High |
| Dexamethasone | High |
| minocycline | High |
| Belnacasan (VX-765) | High |
| Pralnacasan (VX-740, HMR3480) | High |
| cinnamic aldehyde | High |
| Dimethyl fumarate | High |
| curcumin | High |
| iguratimod | High |
| (-)-Epigallocatechin gallate | High |
| TAK-242 | High |
| IRAK4 inhibitors | High |
| Dehydroxymethylepoxyquinomicin (DHMEQ) | High |

## Overall Assessment of the AOP

### Domain of Applicability

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Not Otherwise Specified | High |

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

Although sex differences in immune responses are well known (Klein and Flanagan, 2016), there is no reports regarding the sex difference in IL-1 production, IL-1 function or susceptibility to infection as adverse effect of IL-1 blocking agent. Again, age-dependent difference in IL-1 signaling is not known.

The IL1B gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, and frog (https://www.ncbi.nlm.nih.gov/homologene/481), and the Myd88 gene is conserved in human, chimpanzee, Rhesus monkey, dog, cow, rat, chicken, zebrafish, mosquito, and frog (https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=1849).

The NFKB1 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, and frog.

275 organisms have orthologs with human gene NFKB1.

(https://www.ncbi.nlm.nih.gov/gene/4790)

The lower level of stress-induced IL-1b expression is demonstrated in the aged murine keratinocytes (Pilkington et al., 2018).

The IL-1b production by mouse oral mucosal leukocytes stimulated with candida albicans was reduced with aging (Bhaskaran et al., 2020).

The baseline IL-1 signaling of the upper respiratory tract lavage was reduced in murine newborn mice (Kuipers et al., 2018).

## Essentiality of the Key Events

The experiments using knockout mice revealed that the deficiency of IL-1 signaling led to bacterial, tuberculosis or viral infection (Bohrer et al., 2018; Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Labow et al., 1997; Tian, Jin and Dubin, 2017; Yamada et al., 2000).

IL-1 receptor antagonist IL-1Ra was purified in 1990, and the cDNA reported that same year. IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (generic anakinra) is fully active in blocking the IL-1R1, and therefore, the activities of IL-1α and IL-1β. Anakinra is approved for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndrome (CAPS). Since its introduction in 2002 for the treatment of rheumatoid arthritis, anakinra has had a remarkable record of safety. However, Fleischmann et al. (Fleischmann et al., 2003) reported that serious infectious episodes were observed more frequently in the anakinra group (2.1% versus 0.4% in the placebo group) and other authors reported the increased susceptibility to bacterial or tuberculosis infection (Genovese et al., 2004; Kullenberg et al., 2016; Lequerre et al., 2008). As IL-1 signaling antagonists, two drugs went up to the market, canakinumab (anti-IL-1b antibody) and rilonacept (soluble IL-1R). Several reports described that the administration of these drugs led to immunosuppression or increased susceptibility to infection (De Benedetti et al., 2018; Imagawa et al., 2013; Lachmann et al., 2009; Schlesinger et al., 2012).

In a similar way, defect of MyD88 signaling caused by knockout of mice gene or deficiency in human patient leads to the increased susceptibility to bacterial or tuberculosis infection (von Bernuth et al., 2012).

Mice lacking NF-kB p50 are unable effectively to clear L. monocytogenes and are more susceptible to infection with S. peumoniae (Sha et al., 1995).

## Weight of Evidence Summary

The recent review of IL-1 pathway by Weber et al. (Weber, Wasiliew and Kracht, 2010a) has clearly described the intracellular signaling event from the binding of IL-1α or IL-1β to IL-1R to the activation of NF-κB through the assemble of MyD88 to the trimeric complex composed of IL-1, IL-R1, and IL-1RacP. The sequentiality and essentiality of each signaling molecule have been demonstrated by mice lacking relevant molecules (Dinarello, 2018; Weber, Wasiliew and Kracht, 2010a, b).

There were several reports that described that administration of IL-1R antagonist or neutralizing antibody led to the suppression of downstream phenomena, which included internalization of IL-1 (Dripps et al., 1991), production of $PGE_2$ (Hannum et al., 1990; Seckinger, Kaufmann and Dayer, 1990), IL-6 (Goh et al., 2014), and T cell proliferation (Seckinger, Kaufmann and Dayer, 1990).

Several reports described that the administration of IL-1 receptor antagonist IL-1Ra , canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) led to increased susceptibility to infection (De Benedetti et al., 2018; Fleischmann et al., 2003; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Schlesinger et al., 2012; Yokota et al., 2017). In addition to these human data, the experiments using knockout mice revealed that the lack of IL-1 signaling led to bacterial, tuberculosis or viral infection(Bohrer et al., 2018; Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Labow et al., 1997; Tian, Jin and Dubin, 2017; Yamada et al., 2000). Moreover, polymorphism of IL-1b or IL-1Ra leads to the increased susceptibility to tuberculosis, severe sepsis or fungal infection (Fang et al., 1999; Motsinger-Reif et al., 2010; Wojtowicz et al., 2015).

**Biological plausibility**

Inhibition of IL-1 binding to IL-1 receptor leads to Inhibition, Nuclear factor kappa B (NF-kB)

IL-1α and IL-1β independently bind the type I IL-1 receptor (IL-1R1), which is ubiquitously expressed. The IL-1R3 (formerly IL-1R accessory protein (IL-1RAcP)) serves as a co-receptor that is required for signal transduction of IL-1/IL-1RI complexes.

The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1RI that facilitates recruitment of IL-1R3. the trimeric complex rapidly assembles two intracellular signaling proteins, myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor–activated protein kinase (IRAK) 4. This is paralleled by the (auto)phosphorylation of IRAK4, which subsequently phosphorylates IRAK1 and IRAK2, and then this is followed by the recruitment and oligomerization of tumor necrosis factor–associated factor (TRAF) 6. Activation of NF-κB by IL-1 requires the activation of inhibitor of nuclear factor B (IκB) kinase 2 (IKK2). Activated IKK phosphorylates IκBα, which promotes its K48-linked polyubiquitination and subsequent degradation by the proteasome. IκB destruction allows the release of p50 and p65 NF-κB

subunits and their nuclear translocation, which is the central step in activation of NF-κB. Both NF-κBs bind to a conserved DNA motif that is found in numerous IL-1–responsive genes (Weber, Wasiliew and Kracht, 2010a, b).

Inhibition, Nuclear factor kappa B (NF-κB) leads to Suppression of T cell activation

In T lineage cells, the temporal regulation of NF-κB controls the stepwise differentiation and antigen-dependent selection of conventional and specialized subsets of T cells in response to T cell receptor and costimulatory, cytokines and growth factor signals. Cytokines include cytokines produced from macrophage or monocyte such as IL-1β (Gerondakis et al., 2014).

Suppression of T cell activation leads to supression of TDAR

T cell-derived cytokines play important roles in TDAR. Among them, IL-2 promotes proliferation of B cells, and IL-4 affects maturation and class switching of B cells as well as proliferation.

Th2 cells produce cytokines including IL-4. Suplatast tosilate (IPD) is known as an inhibitor of the production of IL-4 and IL-5 in Th2 cells and reduces the production of antigen specific IgE in human cell culture and mice (Taiho Pharmaceutical 2013). These findings suggests that the reduction of IL-4 production by the inhibitor of

Th2 cell cytokines results in reduced production of IgE and/or IgG1 through inhibitions of maturation, proliferation and class switching of B cells.

IL-2 binds to IL-2 receptor (IL-2R) and acts on T cells. CD25 is one the of IL-2R. Basiliximab (Simulect) is known as anti-CD25antibody. Basiliximab binds to IL-2R and blocks IL-2 signaling. Clinical transplantation study of basiliximab reveals decreases in rejections. On the other hand, basiliximab inhibits the activation of

antigen specific T cells (Novartis Pharma 2016).

Based on these evidences, the insufficient T cell or B cell function causes suppression of TDAR

**Empirical support**

1. **Impaired IL-1R signaling.**

Decreased production of IL-1 or inhibition of the binding of IL-1 to IL-1R impair IL-1R signaling.

    1. **Decreased IL-1 production**

Decreased IL-1 production by macrophages or dendritic cells can be induced by suppressed IL-1β mRNA induction or suppressed maturation of pro-IL-1β. Dexamethasone is one of the representative drugs that significantly suppress IL-1β production from monocytes (Finch-Arietta and Cochran, 1991). Other than dexamethasone, the inhibition of various targets in different layers from the stimulation of PRRs or the receptors of proinflammatory cytokines to the activation of NF-κB or the inhibition of posttranscriptional regulation of pro-IL-1β cause impaired decreased IL-1β production.

Quite a few compounds have been reported to inhibit NF-κB signaling by several different mechanisms reviewed by Fuchs (Fuchs, 2010). In fact, dimethyl fumarate inhibits the activation of NF-κB, resulting in a loss of proinflammatory cytokine production, distorted maturation and function of antigen-presenting cells, and immune deviation of T helper cells (Th) from the type 1 (Th1) and type 17 (Th17) profiles to a type 2 (Th2) phenotype (McGuire et al., 2016; Peng et al., 2012). Several studies have shown intriguing pharmacologic effects associated with curcumin, which inhibits NF-κB expression by regulating NF-κB/IκB pathway and down-regulates expression of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNFα (Wang et al., 2018). Iguratimod, a methanesulfonanilide, that is a novel disease-modifying antirheumatic drug, inhibits NF-κB but not its inhibitor, IκBα, and inhibits the production of IL-1b (Mucke, 2012). Epigalocathechin gallate (EGCG) has been reported to inhibit NF-κB activation through inhibition of p65 phosphorylation (Wheeler et al., 2004) and suppress the production of LPS-stimulated IL-1b (Wang et al., 2020). DHMEQ inhibits LPS-induced NF-κB activation by inhibiting its nuclear translocation from the cytoplasm. It also inhibits LPS-induced secretion of IL-1b (Suzuki and Umezawa, 2006).

Other than the inhibitors for NF-κB signaling, which can be stimulated by various stimulations other than TLR4 stimulation, there are signaling molecules that are specific to TLR4 signaling, such as TLR4, Mal, TRAM, Myd88, IRAK4, and IRAK1/2 (Vallabhapurapu and Karin, 2009). There are several chemicals that targe some of these molecules, an inhibitors of TLR4 such as TAK-242 (Matsunaga et al., 2011) and various IRAK4 inhibitors (Lee et al., 2017). IRAK4 has recently attracted attention as a therapeutic target for inflammation and tumor diseases (Chaudhary, Robinson and Romero, 2015).

Beside transcriptional regulation of IL-1b production, minocycline, and two prodrugs, pralnacasan (VX-740) and belnacasan (VX-765) that are orally absorbed and converted into the active principle, VRT-018858 and VRT-043198, respectively (Fenini, Contassot and French, 2017) suppress IL-1 signaling by the inhibition of caspase-1 activation. Caspase-1 is an essential enzyme for maturation of pro- IL-1β and the secretion of mature IL-1β (Vincent and Mohr, 2007). Recently, it has been reported that cinnamic aldehyde suppresses serum IL-1β level in endotoxin poisoning mice (Xu et al., 2017).

1-2. **Blocking of binding of IL-1 to IL-1R1**

IL-1α and IL-1β independently bind the type I IL-1 receptor (IL-1R1), which is ubiquitously expressed. IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (anakinra) is fully active in blocking the IL-1R1, and therefore, the biological activities of IL-1α and IL-1β. The binding of IL-1α and IL-1β to IL-1R1 can be suppressed by soluble IL-1R like rilonacept (Kapur and Bonk, 2009). The binding of IL-1β to IL-1R1 can be inhibited by anti-IL-1β antibody (canakinumab and gevokizumab) (Church and McDermott, 2009) (Roell et al., 2010).

Several reports described that the administration of IL-1 receptor antagonist IL-1Ra , canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) led to increased susceptibility to infection (De Benedetti et al., 2018; Fleischmann et al., 2003; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Schlesinger et al., 2012; Yokota et al., 2017).

2. **Immunosuppression by impaired IL-1 receptor signaling**

In addition to these human data, the experiments using knockout mice revealed that the lack of IL-1 signaling either by the lack of IL-1a or IL-1b or the lack of IL-1 receptor led to bacterial, tuberculosis or viral infection(Bohrer et al., 2018; Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Labow et al., 1997; Tian, Jin and Dubin, 2017; Yamada et al., 2000). Moreover, polymorphism of IL-1b or IL-1Ra leads to the increased susceptibility to tuberculosis, severe sepsis or fungal infection (Fang et al., 1999; Motsinger-Reif et al., 2010; Wojtowicz et al., 2015).

### Quantitative Consideration

**IL-1Ra blocks IL-1 signaling:**

IL-lra alone at concentrations as high as 1 mg/mL did not induce IL-la, IL-lβ, TNFa, or IL-6 synthesis. Suppression of IL-1-induced IL-1, TNFa, or IL-6 synthesis was dose-dependent (P ≦ .0001). At a twofold molar excess, IL-lra inhibited IL-1-induced IL-1 or TNFa synthesis by 50% (P < .01); an equimolar concentration of IL-lra inhibited synthesis of these two cytokines by over 20% (P < .05). A 10-fold molar excess of IL-lra over IL-lβ reduced IL-lβ-induced IL-la by 95% (P = .01) and IL-la-induced IL-1β by 73% (P < .01). In elutriated monocytes, a 10-fold molar excess of IL-lra reduced IL-lβ-induced IL-la by 82% (P < .05), TNFa by 64% (P = .05), and IL-6 by 47% (P < .05). (Granowitz et al., 1992)

**Canakinumab (ACZ885, Ilaris):**

The antibody binds to human IL-1β with high affinity (about 40 pmol/l). The antibody was found to neutralize the bioactivity of human IL-1β on primary human fibroblasts in vitro 44.6 pmol/l (7.1 ± 0.56 ng/ml; n = 6) of ED50. Application of Canakinumab intraperitoneally 2 hours before injecting the IL-1β producing cells completely suppressed joint swelling (0.06 mg/kg of EC50) (Alten et al., 2008).

Primary human fibroblasts are stimulated with recombinant IL-1b or conditioned medium obtained from LPS-stimulated human PBMCs in the presence of various concentrations of Cankinumab or IL-1RA ranging from 6 to 18,000 pM. Supernatant is taken after 16 h stimulation and assayed for IL-6 by ELISA. Canakinumab typically have 1 nM or less of EC50 for inhibition of IL-6 production (Canakinumab Patent Application WO02/16436.)

**Rilonacept (IL-1 Trap, Arcalyst):**

Incubation of the human MRC5 fibroblastic cell line with IL-1β induces secretion of IL-6. At a constant amount of IL-1β (4 pM), the IC50 of the IL-1 trap is ~2 pM. Another unique property of the IL-1 trap is that it not only blocks IL-1β, but also blocks IL-1α with high affinity (KD = ~3 pM; data not shown). The titration curve of IL-1 trap in the presence of 10 pM IL-1β shows an IC50 of 6.5 pM, which corresponds to a calculated KD of 1.5 pM (This affinity is 100 times higher than that of the soluble single component receptor IL-1RI (Economides et al., 2003).

## Considerations for Potential Applications of the AOP (optional)

The impaired IL-1 signaling can lead to immunosuppression. Therefore, the test guideline to detect chemicals that decrease IL-1 signaling is required to support regulatory decision-making. This AOP can promote the understanding of the usefulness of the test guideline.

## References

Alten, R., Gram, H., Joosten, L.A., et al. (2008), The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. *Arthritis Res Ther* 10: R67, 10.1186/ar2438

Bent, R., Moll, L., Grabbe, S., et al. (2018), Interleukin-1 Beta-A Friend or Foe in Malignancies? *Int J Mol Sci* 19, 10.3390/ijms19082155

Bhaskaran, N., Faddoul, F., Paes da Silva, A., et al. (2020), IL-1beta-MyD88-mTOR Axis Promotes Immune-Protective IL-17A(+)Foxp3(+) Cells During Mucosal Infection and Is Dysregulated With Aging. *Front Immunol* 11: 595936, 10.3389/fimmu.2020.595936

Bohrer, A.C., Tocheny, C., Assmann, M., et al. (2018), Cutting Edge: IL-1R1 Mediates Host Resistance to Mycobacterium tuberculosis by Trans-Protection of Infected Cells. *J Immunol* 201: 1645-1650, 10.4049/jimmunol.1800438

Chaudhary, D., Robinson, S., Romero, D.L. (2015), Recent advances in the discovery of small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) as a therapeutic target for inflammation and oncology disorders. *J Med Chem* 58: 96-110, 10.1021/jm5016044

Church, L.D., McDermott, M.F. (2009), Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther* 11: 81-89,

De Benedetti, F., Gattorno, M., Anton, J., et al. (2018), Canakinumab for the Treatment of Autoinflammatory Recurrent Fever Syndromes. *N Engl J Med* 378: 1908-1919, 10.1056/NEJMoa1706314

Dinarello, C.A. (2018), Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev* 281: 8-27, 10.1111/imr.12621

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., et al. (1991), Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266: 10331-10336,

Economides, A.N., Carpenter, L.R., Rudge, J.S., et al. (2003), Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat Med* 9: 47-52, 10.1038/nm811

Fang, X.M., Schroder, S., Hoeft, A., et al. (1999), Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis. *Crit Care Med* 27: 1330-1334, 10.1097/00003246-199907000-00024

Fenini, G., Contassot, E., French, L.E. (2017), Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. *Front Pharmacol* 8: 278, 10.3389/fphar.2017.00278

Finch-Arietta, M.B., Cochran, F.R. (1991), Cytokine production in whole blood ex vivo. *Agents Actions* 34: 49-52, 10.1007/BF01993235

Fleischmann, R.M., Schechtman, J., Bennett, R., et al. (2003), Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: A large, international, multicenter, placebo-controlled trial. *Arthritis Rheum* 48: 927-934, 10.1002/art.10870

Fuchs, O. (2010), Transcription factor NF-kappaB inhibitors as single therapeutic agents or in combination with classical chemotherapeutic agents for the treatment of hematologic malignancies. *Curr Mol Pharmacol* 3: 98-122, 10.2174/1874467211003030098

Genovese, M.C., Cohen, S., Moreland, L., et al. (2004), Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. *Arthritis Rheum* 50: 1412-1419, 10.1002/art.20221

Gerondakis, S., Fulford, T.S., Messina, N.L., et al. (2014), NF-kappaB control of T cell development. *Nat Immunol* 15: 15-25, 10.1038/ni.2785

Goh, A.X., Bertin-Maghit, S., Ping Yeo, S., et al. (2014), A novel human anti-interleukin-1beta neutralizing monoclonal antibody showing in vivo efficacy. *MAbs* 6: 765-773, 10.4161/mabs.28614

Granowitz, E.V., Clark, B.D., Vannier, E., et al. (1992), Effect of interleukin-1 (IL-1) blockade on cytokine synthesis: I. IL-1 receptor antagonist inhibits IL-1-induced cytokine synthesis and blocks the binding of IL-1 to its type II receptor on human monocytes. *Blood* 79: 2356-2363,

Guler, R., Parihar, S.P., Spohn, G., et al. (2011), Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. *Vaccine* 29: 1339-1346, 10.1016/j.vaccine.2010.10.045

Handa, P., Vemulakonda, A., Kowdley, K.V., et al. (2016), Mitochondrial DNA from hepatocytes as a ligand for TLR9: Drivers of nonalcoholic steatohepatitis? *World J Gastroenterol* 22: 6965-6971, 10.3748/wjg.v22.i31.6965

Hannum, C.H., Wilcox, C.J., Arend, W.P., et al. (1990), Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343: 336-340, 10.1038/343336a0

Horino, T., Matsumoto, T., Ishikawa, H., et al. (2009), Interleukin-1 deficiency in combination with macrophage depletion increases susceptibility to Pseudomonas aeruginosa bacteremia. *Microbiol Immunol* 53: 502-511, 10.1111/j.1348-0421.2009.00143.x

Imagawa, T., Nishikomori, R., Takada, H., et al. (2013), Safety and efficacy of canakinumab in Japanese patients with phenotypes of cryopyrin-associated periodic syndrome as established in the first open-label, phase-3 pivotal study (24-week results). *Clin Exp Rheumatol* 31: 302-309,

Jain, A., Kaczanowska, S., Davila, E. (2014), IL-1 receptor-associated kinase signaling and its role in inflammation, cancer, progression, and therapy resistance. *Frontiers in Immunology* 5:553.

Juffermans, N.P., Florquin, S., Camoglio, L., et al. (2000), Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J Infect Dis* 182: 902-908, 10.1086/315771

Kapur, S., Bonk, M.E. (2009), Rilonacept (arcalyst), an interleukin-1 trap for the treatment of cryopyrin-associated periodic syndromes. *P t* 34: 138-141,

Klein, S.L., Flanagan, K.L. (2016), Sex differences in immune responses. *Nat Rev Immunol* 16: 626-638, 10.1038/nri.2016.90

Kuipers, K., Lokken, K.L., Zangari, T., et al. (2018), Age-related differences in IL-1 signaling and capsule serotype affect persistence of Streptococcus pneumoniae colonization. *PLoS Pathog* 14: e1007396, 10.1371/journal.ppat.1007396

Kullenberg, T., Lofqvist, M., Leinonen, M., et al. (2016), Long-term safety profile of anakinra in patients with severe cryopyrin-associated periodic syndromes. *Rheumatology (Oxford)* 55: 1499-1506, 10.1093/rheumatology/kew208

Labow, M., Shuster, D., Zetterstrom, M., et al. (1997), Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J Immunol* 159: 2452-2461,

Lachmann, H.J., Kone-Paut, I., Kuemmerle-Deschner, J.B., et al. (2009), Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med* 360: 2416-2425, 10.1056/NEJMoa0810787

Lee, K.L., Ambler, C.M., Anderson, D.R., et al. (2017), Discovery of Clinical Candidate 1-{[(2S,3S,4S)-3-Ethyl-4-fluoro-5-oxopyrrolidin-2-yl]methoxy}-7-methoxyisoquinoli ne-6-carboxamide (PF-06650833), a Potent, Selective Inhibitor of Interleukin-1 Receptor Associated Kinase 4 (IRAK4), by Fragment-Based Drug Design. *J Med Chem* 60: 5521-5542, 10.1021/acs.jmedchem.7b00231

Lequerre, T., Quartier, P., Rosellini, D., et al. (2008), Interleukin-1 receptor antagonist (anakinra) treatment in patients with systemic-onset juvenile idiopathic arthritis or adult onset Still disease: preliminary experience in France. *Ann Rheum Dis* 67: 302-308, 10.1136/ard.2007.076034

Matsunaga, N., Tsuchimori, N., Matsumoto, T., et al. (2011), TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. *Mol Pharmacol* 79: 34-41, 10.1124/mol.110.068064

McGuire, V.A., Ruiz-Zorrilla Diez, T., Emmerich, C.H., et al. (2016), Dimethyl fumarate blocks pro-inflammatory cytokine production via inhibition of TLR induced M1 and K63 ubiquitin chain formation. *Sci Rep* 6: 31159, 10.1038/srep31159

Motsinger-Reif, A.A., Antas, P.R., Oki, N.O., et al. (2010), Polymorphisms in IL-1beta, vitamin D receptor Fok1, and Toll-like receptor 2 are associated with extrapulmonary tuberculosis. *BMC Med Genet* 11: 37, 10.1186/1471-2350-11-37

Mucke, H.A. (2012), Iguratimod: a new disease-modifying antirheumatic drug. *Drugs Today (Barc)* 48: 577-586, 10.1358/dot.2012.48.9.1855758

Newton, K., Dixit, V.M. (2012), Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol* 4, 10.1101/cshperspect.a006049

Novartis Pharma K.K. (2016). Drug interview form Simulect i.v. injection 20 mg. 10th edition.

Peng, H., Guerau-de-Arellano, M., Mehta, V.B., et al. (2012), Dimethyl fumarate inhibits dendritic cell maturation via nuclear factor kappaB (NF-kappaB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. *J Biol Chem* 287: 28017-28026, 10.1074/jbc.M112.383380

Pilkington, S.M., Ogden, S., Eaton, L.H., et al. (2018), Lower levels of interleukin-1beta gene expression are associated with impaired Langerhans' cell migration in aged human skin. *Immunology* 153: 60-70, 10.1111/imm.12810

Roell, M.K., Issafras, H., Bauer, R.J., et al. (2010), Kinetic approach to pathway attenuation using XOMA 052, a regulatory therapeutic antibody that modulates interleukin-1beta activity. *J Biol Chem* 285: 20607-20614, 10.1074/jbc.M110.115790

Schlesinger, N., Alten, R.E., Bardin, T., et al. (2012), Canakinumab for acute gouty arthritis in patients with limited treatment options: results from two randomised, multicentre, active-controlled, double-blind trials and their initial extensions. *Ann Rheum Dis* 71: 1839-1848, 10.1136/annrheumdis-2011-200908

Seckinger, P., Kaufmann, M.T., Dayer, J.M. (1990), An interleukin 1 inhibitor affects both cell-associated interleukin 1-induced T cell proliferation and PGE2/collagenase production by human dermal fibroblasts and synovial cells. *Immunobiology* 180: 316-327, 10.1016/s0171-2985(11)80295-0

Sha, W.C., Liou, H.C., Tuomanen, E.I., et al. (1995), Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 80: 321-330,

Soares, M.P., Teixeira, L., Moita, L.F. (2017), Disease tolerance and immunity in host protection against infection. *Nat Rev Immunol* 17: 83-96, 10.1038/nri.2016.136

Suzuki, E., Umezawa, K. (2006), Inhibition of macrophage activation and phagocytosis by a novel NF-kappaB inhibitor, dehydroxymethylepoxyquinomicin. *Biomed Pharmacother* 60: 578-586, 10.1016/j.biopha.2006.07.089

Taiho Pharmaceutical Co.,Ltd. (2013) Drug interview form IPD capsule 50 and 100. Revised 5th edition.

Tian, T., Jin, M.Q., Dubin, K. (2017), IL-1R Type 1-Deficient Mice Demonstrate an Impaired Host Immune Response against Cutaneous Vaccinia Virus Infection. 198: 4341-4351, 10.4049/jimmunol.1500106

Vallabhapurapu, S., Karin, M. (2009), Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* 27: 693-733, 10.1146/annurev.immunol.021908.132641

Vincent, J.A., Mohr, S. (2007), Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. *Diabetes* 56: 224-230, 10.2337/db06-0427

von Bernuth, H., Picard, C., Puel, A., et al. (2012), Experimental and natural infections in MyD88- and IRAK-4-deficient mice and humans. *Eur J Immunol* 42: 3126-3135, 10.1002/eji.201242683

Wang, F., Han, Y., Xi, S., et al. (2020), Catechins reduce inflammation in lipopolysaccharide-stimulated dental pulp cells by inhibiting activation of the NF-kappaB pathway. *Oral Dis* 26: 815-821, 10.1111/odi.13290

Wang, Y., Tang, Q., Duan, P., et al. (2018), Curcumin as a therapeutic agent for blocking NF-kappaB activation in ulcerative colitis. *Immunopharmacol Immunotoxicol* 40: 476-482, 10.1080/08923973.2018.1469145

Weber, A., Wasiliew, P., Kracht, M. (2010a), Interleukin-1 (IL-1) pathway. *Sci Signal* 3: cm1, 10.1126/scisignal.3105cm1

Weber, A., Wasiliew, P., Kracht, M. (2010b), Interleukin-1beta (IL-1beta) processing pathway. *Sci Signal* 3: cm2, 10.1126/scisignal.3105cm2

Wheeler, D.S., Catravas, J.D., Odoms, K., et al. (2004), Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr* 134: 1039-1044, 10.1093/jn/134.5.1039

Wojtowicz, A., Gresnigt, M.S., Lecompte, T., et al. (2015), IL1B and DEFB1 Polymorphisms Increase Susceptibility to Invasive Mold Infection After Solid-Organ Transplantation. *J Infect Dis* 211: 1646-1657, 10.1093/infdis/jiu636

Xu, F., Wang, F., Wen, T., et al. (2017), Inhibition of NLRP3 inflammasome: a new protective mechanism of cinnamaldehyde in endotoxin poisoning of mice. *Immunopharmacol Immunotoxicol* 39: 296-304, 10.1080/08923973.2017.1355377

Yamada, H., Mizumo, S., Horai, R., et al. (2000), Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. *Lab Invest* 80: 759-767,

Yang, Han, Z., Oppenheim, J.J. (2017), Alarmins and immunity. *Immunol Rev* 280: 41-56, 10.1111/imr.12577

Yokota, S., Imagawa, T., Nishikomori, R., et al. (2017), Long-term safety and efficacy of canakinumab in cryopyrin-associated periodic syndrome: results from an open-label, phase III pivotal study in Japanese patients. *Clin Exp Rheumatol* 35 Suppl 108: 19-26,

# Appendix 1

## List of MIEs in this AOP

**Event: 1700: Impaired IL-1R1 signaling in T cell**

### Short Name: Impaired IL-1R1 signaling

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:277 - Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | MolecularInitiatingEvent |

### Stressors

| Name |
|---|
| IL-1 receptor antagonist IL-1Ra (Anakinra) |
| anti-IL-1b antibody (Canakinumab) |
| soluble IL-1R (Rilonacept) |

| Name |
| --- |
| curcumin |
| iguratimod |
| epigallocathechin gallate |
| TAK-242 |
| IRAK4 inhibitors |
| Dehydroxymethylepoxyquinomicin (DHMEQ) |
| Dimethyl fumarate |
| anti-IL-1b antibody (Gevokizumab) |

### Biological Context

| Level of Biological Organization |
| --- |
| Molecular |

### Cell term

| Cell term |
| --- |
| T cell |

### Organ term

| Organ term |
| --- |
| immune system |

## Evidence for Perturbation by Stressor

### Overview for Molecular Initiating Event

Dex inhibits IL-1β gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-κB/Rel and AP-1 activation (Jeon et al., 2000).

Dex suppresses LPS-induced gene expression of IL-1β in rat lung. (in vivo) (Qiu et al., 1997)

Dex inhibits the release of IL-1β by human leukocyte stimulated with Streptococcus pneumoniae stimulation (van Furth et al., 1995).

Treatment of peripheral blood monocytes with 2 mg/ml LPS potently increased IL-1β release (p= 0.001) and Dex ($10^{-7}$ M) significantly reduced both resting and stimulated IL-1β release (p 0.009).) (Morand, Rickard and Goulding, 1993)

Dex effectively blocks the glutamine antagonist acivicin-induced expression of IL-1β mRNA by HL-60 leukemia cells (Weinberg, Mason and Wortham, 1992).

LPS treatment induced a significant upregulation of the mRNA and release of IL-1β from retinal microglia. Minocycline inhibited its releases. Thus, minocycline might exert its anti-inflammatory effect on microglia by inhibiting the expression and release of IL-1β (Wang et al., 2005).

Caspase-1 inhibition reduced the release of IL-1β in organotypic slices exposed to LPS+ATP. Administration of pralnacasan (intracerebroventricular, 50 μg) or belnacasan (intraperitoneal, 25–200 mg/kg) to rats blocked seizure-induced production of IL-1β in the hippocampus, and resulted in a twofold delay in seizure onset and 50% reduction in seizure duration (Ravizza et al., 2006).

Belnacasan, an orally active IL-1β converting enzyme/caspase-1 inhibitor, blocked IL-1β secretion with equal potency in LPS-stimulated cells from familial cold urticarial associated syndrome and control subjects (Stack et al., 2005).

In LPS-induced acute lung injury (ALI) mice model, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were

significantly decreased by cinnamaldehyde (CA) (Huang and Wang, 2017).

The suppressing capacities of six cinnamaldehyde-related compounds were evaluated and compared by using the LPS-primed and ATP-activated macrophages. At concentrations of 25~100  M, cinnamaldehyde and 2-methoxy cinnamaldehyde dose-dependently inhibited IL-1β secretion (Ho, Chang and Chang, 2018).

In vitro, CA decreased the levels of pro-IL-1β and IL-1β in cell culture supernatants, as well as the expression of NLRP3 and IL-1β mRNA in cells. In vivo, CA decreased IL-1β production in serum. Furthermore, CA suppressed LPS-induced NLRP3, p20, Pro-IL-1β, P2X7 receptor (P2X7R) and cathepsin B protein expression in lung, as well as the expression of NLRP3 and IL-1β mRNA (Xu et al., 2017).

IL-1 is known to mediates autoinflammatory syndrome, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever. Blocking of binding of IL-1 to IL-1R1 by anakinra, canakinumab, and rilonacept have been already used to treat these autoinflammatory syndrome associated with overactivation of IL-1 signaling (Quartier, 2011).

Various inhibitors for NF-κB, such as dimethyl fumarate, curcumin, iguratimod, epigalocathechin gallate (EGCG), and DHMEQ inhibits LPS-induced NF-κB activation and LPS-induced secretion of IL-1b (McGuire et al., 2016; Mucke, 2012; Peng et al., 2012; Suzuki and Umezawa, 2006; Wang et al., 2020; Wang et al., 2018; Wheeler et al., 2004).

Several chemicals that targe some of these molecules, an inhibitors of TLR4 such as TAK-242 (Matsunaga et al., 2011) and various IRAK4 inhibitors (Lee et al., 2017). IRAK4 has recently attracted attention as a therapeutic target for inflammation and tumor diseases.

IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (anakinra) is fully active in blocking the IL-1R1, and therefore, the biological activities of IL-1α and IL-1β. The binding of IL-1α and IL-1β to IL-1R1 can be suppressed by soluble IL-1R like rilonacept (Kapur and Bonk, 2009). The binding of IL-1β to IL-1R1 can be inhibited by anti-IL-1β antibody (canakinumab and gevokizumab) (Church and McDermott, 2009) (Roell et al., 2010).

Various IRAK4 inhibitors are currently under the investigation on the possibility of clinical use for autoimmune disorders (Chaudhary, Robinson and Romero, 2015).

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Although sex differences in immune responses are well known (Klein and Flanagan, 2016), there is no reports regarding the sex difference in IL-1 production, IL-1 function or susceptibility to infection as adverse effect of IL-1 blocking agent. Age-dependent difference in IL-1 signaling is not known.

The IL1B gene is conserved in chimpanzee, rhesus monkey, dog, cow, mouse, rat, and frog (https://www.ncbi.nlm.nih.gov/homologene/481), and the Myd88 gene is conserved in human, chimpanzee, rhesus monkey, dog, cow, rat, chicken, zebrafish, mosquito, and frog (https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=1849).

The lower level of stress-induced IL-1β expression is demonstrated in the aged murine keratinocytes (Pilkington et al., 2018).

The IL-1β production by mouse oral mucosal leukocytes stimulated with candida albicans was reduced with aging (Bhaskaran et al., 2020).

The baseline IL-1 signaling of the upper respiratory tract lavage was reduced in murine newborn mice (Kuipers et al., 2018).

## Key Event Description

1. **Decreased IL-1 production**

Decreased IL-1 production by macrophages or dendritic cells can be induced by suppressed IL-1β mRNA induction or suppressed maturation of pro-IL-1β. Dexamethasone is one of the representative drugs that significantly suppress IL-1β production from monocytes (Finch-Arietta and Cochran, 1991). Other than dexamethasone, the inhibition of various targets in different layers from the stimulation of PRRs or the receptors of proinflammatory cytokines lto the activation of NF-κB or the inhibition of posttranscriptional regulation of pro-IL-1β cause impaired IL-1R1 signaling. Among various PRRs, the signaling through TLR4 is best characterized. In addition, it is beyond the scope of this AOP to cover all signaling through each PRR. So, this AOP focuses on TLR4 signaling.

Lipopolysaccharide (LPS) from the bacteria binds to TLR4 in complex with myeloid differentiation factor-2 (MD2), and this complex initiates signalling by recruiting the adaptor proteins MyD88, TIR domain containing adaptor protein (TIRAP), TIR-domain-containing adapter-inducing interferon-β (TRIF) and TIR-domain containing adaptor (TRAM). MYD88 associates with IL-1R-associated kinase 1 (IRAK1) and IRAK4 and recruits TNFR-associated factor 6 (TRAF6). This complex recruits TGF-β-activated kinase 1 (TAK1), leading to phosphorylation of NF-κB inhibitor (IκB), activation of nuclear factor-κB (NF-κB) and consequent transcription of a range of genes coding for pro-inflammatory cytokines, including tumour necrosis factor (TNF), IL-6, pto-IL-1b, and pro-IL-18 (Mills, 2011).

Therefore, chemicals that affect the signaling pathway leading to the activation of these transcription factors are supposed to suppress IL-1β production. Among them, the chemical substances that affect NF-κB signaling have been investigated most thoroughly. Quite a few compounds have been reported to inhibit NF-κB signaling by several different mechanisms reviewed by Fuchs (Fuchs, 2010). In fact, dimethyl fumarate inhibits the activation of NF-κB, resulting in a loss of proinflammatory cytokine production, distorted maturation and function of antigen-presenting cells, and immune deviation of T helper cells (Th) from the type 1 (Th1) and type 17 (Th17) profiles to a type 2 (Th2) phenotype (McGuire et al., 2016; Peng et al., 2012). Several studies have shown intriguing pharmacologic effects associated with curcumin, which inhibits NF-κB expression by regulating NF-κB/IκB pathway and down-regulates expression of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNFα (Wang et al., 2018). Iguratimod, a methanesulfonanilide, that is a novel disease-modifying antirheumatic drug, inhibits NF-κB but not its inhibitor, IκBα, and inhibits the production of IL-1β (Mucke, 2012). Epigalocathechin gallate (EGCG) has been reported to inhibit NF-κB activation through inhibition of p65 phosphorylation (Wheeler et al., 2004) and suppress the production of LPS-stimulated IL-1β (Wang et al., 2020).

DHMEQ inhibits lLPS-induced NF-κB activation by inhibiting its nuclear translocation from the cytoplasm. It also inhibits LPS-induced secretion of IL-1β (Suzuki and Umezawa, 2006).

Other than the inhibitors for NF-κB signaling, which can be stimulated by various stimulations other than TLR4 stimulation, there are signaling molecules that are specific to TLR4 signaling, such as TLR4, Mal, TRAM, Myd88, IRAK4, and IRAK1/2 (Vallabhapurapu and Karin, 2009). There are several chemicals that targe some of these molecules, an inhibitors of TLR4 such as TAK-242 (Matsunaga et al., 2011) and various IRAK4 inhibitors (Lee et al., 2017). IRAK4 has recently attracted attention as a therapeutic target for inflammation and tumor diseases.

Beside transcriptional regulation of IL-1β production, minocycline, and two prodrugs, pralnacasan (VX-740) and belnacasan (VX-765) that are orally absorbed and converted into the active principle, VRT-018858 and VRT-043198, respectively (Fenini et al., 2017) suppress IL-1 signaling by the inhibition of caspase-1 activation. Caspase-1 is an essential enzyme for maturation of pro- IL-1β and the secretion of mature IL-1β (Vincent and Mohr, 2007). Recently, it has been reported that cinnamicaldehyde suppresses serum IL-1β level in endotoxin poisoning mice (Xu et al., 2017).

2. **Blocking of binding of IL-1 to IL-1R1**

IL-1α and IL-1β independently bind the type I IL-1 receptor (IL-1R1), which is ubiquitously expressed. IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (anakinra) is fully active in blocking the IL-1R1, and therefore, the biological activities of IL-1α and IL-1β. The binding of IL-1α and IL-1β to IL-1R1 can be suppressed by soluble IL-1R like rilonacept (Kapur and Bonk, 2009). The binding of IL-1β to IL-1R1 can be inhibited by anti-IL-1β antibody (canakinumab and gevokizumab) (Church and McDermott, 2009) (Roell et al., 2010).

This AOP focus on the blocking of binding of IL-1 to IL-1R1, and an inhibition or suppression of IL-1 signaling is out of scope, because the molecular initiating event of IL-1 blocking is simple and appropriate for developing AOP. This AOP is expected to be applicable to any chemicals which bind to IL-1R, although such stressor has not been reported.

### How it is Measured or Detected

1. Real time polymerase chain reaction to measure IL-1α or IL-1β mRNA
2. Enzyme-linked immunosorbent assay (ELISA) to detect IL-1α or IL-1 b protein
3. Competitive inhibition binding experiments using $^{125}$I-IL-1α to type I IL-1R present on EL4 thymoma cells, 3T3 fibroblasts, hepatocytes, and Chinese hamster ovary cells expressing recombinant mouse type I IL-1R (McIntyre et al., 1991; Shuck et al., 1991).
4. Measure the ability of the reagent to neutralize the bioactivity of human IL-1β on primary human fibroblasts in vitro(Alten et al., 2008)

### References

Alten, R., Gram, H., Joosten, L.A., et al. (2008), The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. *Arthritis Res Ther* 10: R67, 10.1186/ar2438

Bhaskaran, N., Faddoul, F., Paes da Silva, A., et al. (2020), IL-1beta-MyD88-mTOR Axis Promotes Immune-Protective IL-17A(+)Foxp3(+) Cells During Mucosal Infection and Is Dysregulated With Aging. *Front Immunol* 11: 595936, 10.3389/fimmu.2020.595936

Chaudhary, D., Robinson, S., Romero, D.L. (2015), Recent advances in the discovery of small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) as a therapeutic target for inflammation and oncology disorders. *J Med Chem* 58: 96-110, 10.1021/jm5016044

Church, L.D., McDermott, M.F. (2009), Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther* 11: 81-89,

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., et al. (1991), Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266: 10331-10336,

Fenini, G., Contassot, E., French, L.E. (2017), Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. *Front Pharmacol* 8: 278, 10.3389/fphar.2017.00278

Finch-Arietta, M.B., Cochran, F.R. (1991), Cytokine production in whole blood ex vivo. *Agents Actions* 34: 49-52, 10.1007/BF01993235

Fuchs, O. (2010), Transcription factor NF-kappaB inhibitors as single therapeutic agents or in combination with classical chemotherapeutic agents for the treatment of hematologic malignancies. *Curr Mol Pharmacol* 3: 98-122, 10.2174/1874467211003030098

Ho, S.C., Chang, Y.H., Chang, K.S. (2018), Structural Moieties Required for Cinnamaldehyde-Related Compounds to Inhibit

Canonical IL-1beta Secretion. *Molecules* 23, 10.3390/molecules23123241

Huang, H., Wang, Y. (2017), The protective effect of cinnamaldehyde on lipopolysaccharide induced acute lung injury in mice. *Cell Mol Biol (Noisy-le-grand)* 63: 58-63, 10.14715/cmb/2017.63.8.13

Jeon, Y.J., Han, S.H., Lee, Y.W., et al. (2000), Dexamethasone inhibits IL-1 beta gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-kappa B/Rel and AP-1 activation. *Immunopharmacology* 48: 173-183, 10.1016/s0162-3109(00)00199-5

Kapur, S., Bonk, M.E. (2009), Rilonacept (arcalyst), an interleukin-1 trap for the treatment of cryopyrin-associated periodic syndromes. *P t* 34: 138-141,

Klein, S.L., Flanagan, K.L. (2016), Sex differences in immune responses. *Nat Rev Immunol* 16: 626-638, 10.1038/nri.2016.90

Kuipers, K., Lokken, K.L., Zangari, T., et al. (2018), Age-related differences in IL-1 signaling and capsule serotype affect persistence of Streptococcus pneumoniae colonization. *PLoS Pathog* 14: e1007396, 10.1371/journal.ppat.1007396

Lee, K.L., Ambler, C.M., Anderson, D.R., et al. (2017), Discovery of Clinical Candidate 1-{[(2S,3S,4S)-3-Ethyl-4-fluoro-5-oxopyrrolidin-2-yl]methoxy}-7-methoxyisoquinoli ne-6-carboxamide (PF-06650833), a Potent, Selective Inhibitor of Interleukin-1 Receptor Associated Kinase 4 (IRAK4), by Fragment-Based Drug Design. *J Med Chem* 60: 5521-5542, 10.1021/acs.jmedchem.7b00231

Matsunaga, N., Tsuchimori, N., Matsumoto, T., et al. (2011), TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. *Mol Pharmacol* 79: 34-41, 10.1124/mol.110.068064

McGuire, V.A., Ruiz-Zorrilla Diez, T., Emmerich, C.H., et al. (2016), Dimethyl fumarate blocks pro-inflammatory cytokine production via inhibition of TLR induced M1 and K63 ubiquitin chain formation. *Sci Rep* 6: 31159, 10.1038/srep31159

McIntyre, K.W., Stepan, G.J., Kolinsky, K.D., et al. (1991), Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. *J Exp Med* 173: 931-939,

Mills, K.H. (2011), TLR-dependent T cell activation in autoimmunity. *Nat Rev Immunol* 11: 807-822, 10.1038/nri3095

Morand, E.F., Rickard, D., Goulding, N.J. (1993), Lack of involvement of lipocortin 1 in dexamethasone suppression of IL-1 release. *Mediators Inflamm* 2: 49-52, 10.1155/S0962935193000067

Mucke, H.A. (2012), Iguratimod: a new disease-modifying antirheumatic drug. *Drugs Today (Barc)* 48: 577-586, 10.1358/dot.2012.48.9.1855758

Peng, H., Guerau-de-Arellano, M., Mehta, V.B., et al. (2012), Dimethyl fumarate inhibits dendritic cell maturation via nuclear factor kappaB (NF-kappaB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. *J Biol Chem* 287: 28017-28026, 10.1074/jbc.M112.383380

Pilkington, S.M., Ogden, S., Eaton, L.H., et al. (2018), Lower levels of interleukin-1beta gene expression are associated with impaired Langerhans' cell migration in aged human skin. *Immunology* 153: 60-70, 10.1111/imm.12810

Qiu, H.B., Pan, J.Q., Zhao, Y.Q., et al. (1997), Effects of dexamethasone and ibuprofen on LPS-induced gene expression of TNF alpha, IL-1 beta, and MIP-1 alpha in rat lung. *Zhongguo Yao Li Xue Bao* 18: 165-168,

Quartier, P. (2011), Interleukin-1 antagonists in the treatment of autoinflammatory syndromes, including cryopyrin-associated periodic syndrome. *Open Access Rheumatol* 3: 9-18, 10.2147/oarrr.S6696

Ravizza, T., Lucas, S.M., Balosso, S., et al. (2006), Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. *Epilepsia* 47: 1160-1168, 10.1111/j.1528-1167.2006.00590.x

Roell, M.K., Issafras, H., Bauer, R.J., et al. (2010), Kinetic approach to pathway attenuation using XOMA 052, a regulatory therapeutic antibody that modulates interleukin-1beta activity. *J Biol Chem* 285: 20607-20614, 10.1074/jbc.M110.115790

Shuck, M.E., Eessalu, T.E., Tracey, D.E., et al. (1991), Cloning, heterologous expression and characterization of murine interleukin 1 receptor antagonist protein. *Eur J Immunol* 21: 2775-2780, 10.1002/eji.1830211119

Stack, J.H., Beaumont, K., Larsen, P.D., et al. (2005), IL-converting enzyme/caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. *J Immunol* 175: 2630-2634, 10.4049/jimmunol.175.4.2630

Suzuki, E., Umezawa, K. (2006), Inhibition of macrophage activation and phagocytosis by a novel NF-kappaB inhibitor, dehydroxymethylepoxyquinomicin. *Biomed Pharmacother* 60: 578-586, 10.1016/j.biopha.2006.07.089

Vallabhapurapu, S., Karin, M. (2009), Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* 27: 693-733, 10.1146/annurev.immunol.021908.132641

van Furth, A.M., Seijmonsbergen, E.M., Langermans, J.A., et al. (1995), Effect of xanthine derivates and dexamethasone on

Streptococcus pneumoniae-stimulated production of tumor necrosis factor alpha, interleukin-1 beta (IL-1 beta), and IL-10 by human leukocytes. *Clin Diagn Lab Immunol* 2: 689-692,

Vincent, J.A., Mohr, S. (2007), Inhibition of caspase-1/interleukin-1beta signaling prevents degeneration of retinal capillaries in diabetes and galactosemia. *Diabetes* 56: 224-230, 10.2337/db06-0427

Wang, A.L., Yu, A.C., Lau, L.T., et al. (2005), Minocycline inhibits LPS-induced retinal microglia activation. *Neurochem Int* 47: 152-158, 10.1016/j.neuint.2005.04.018

Wang, F., Han, Y., Xi, S., et al. (2020), Catechins reduce inflammation in lipopolysaccharide-stimulated dental pulp cells by inhibiting activation of the NF-kappaB pathway. *Oral Dis* 26: 815-821, 10.1111/odi.13290

Wang, Y., Tang, Q., Duan, P., et al. (2018), Curcumin as a therapeutic agent for blocking NF-kappaB activation in ulcerative colitis. *Immunopharmacol Immunotoxicol* 40: 476-482, 10.1080/08923973.2018.1469145

Weinberg, J.B., Mason, S.N., Wortham, T.S. (1992), Inhibition of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta) messenger RNA (mRNA) expression in HL-60 leukemia cells by pentoxifylline and dexamethasone: dissociation of acivicin-induced TNF-alpha and IL-1 beta mRNA expression from acivicin-induced monocytoid differentiation. *Blood* 79: 3337-3343,

Wheeler, D.S., Catravas, J.D., Odoms, K., et al. (2004), Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr* 134: 1039-1044, 10.1093/jn/134.5.1039

Xu, F., Wang, F., Wen, T., et al. (2017), Inhibition of NLRP3 inflammasome: a new protective mechanism of cinnamaldehyde in endotoxin poisoning of mice. *Immunopharmacol Immunotoxicol* 39: 296-304, 10.1080/08923973.2017.1355377

## List of Key Events in the AOP

### Event: 202: Inhibition, Nuclear factor kappa B (NF-kB)

### Short Name: Inhibition, Nuclear factor kappa B (NF-kB)

### Key Event Component

| Process | Object | Action |
|---|---|---|
| I-kappaB kinase/NF-kappaB signaling | transcription factor NF-kappa-B subunit | decreased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:14 - Glucocorticoid Receptor Activation Leading to Increased Disease Susceptibility | KeyEvent |
| Aop:278 - IKK complex inhibition leading to liver injury | KeyEvent |
| Aop:277 - Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | KeyEvent |
| Aop:447 - Kidney failure induced by inhibition of mitochondrial electron transfer chain through apoptosis, inflammation and oxidative stress pathways | KeyEvent |

### Stressors

| Name |
|---|
| IL-1 receptor antagonist IL-1Ra (Anakinra) |
| anti-IL-1b antibody (Canakinumab) |
| soluble IL-1R (Rilonacept) |

### Biological Context

**Level of Biological Organization**

Molecular

### Cell term

**Cell term**

T cell

### Organ term

**Organ term**

immune system

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

The binding of sex steroids to their respective steroid receptors directly influences NF-κB signaling, resulting in differential production of cytokines and chemokines (McKay and Cidlowski, 1999; Pernis, 2007). 17b-estradiol regulates pro-inflammatory responses that are transcriptionally mediated by NF-κB through a negative feedback and/or transrepressive interaction with NF-κB (Straub, 2007). Progesterone suppresses innate immune responses and NF-κB signal transduction reviewed by Klein et al. (Klein and Flanagan, 2016). Androgen-receptor signaling antagonises transcriptional factors NF-κB(McKay and Cidlowski, 1999).

**Evidence for perturbation of this molecular initiating event by stressor**

Dex inhibits IL-1β gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-κB/Rel and AP-1 activation (Jeon et al., 2000).

Various inhibitors for NF-κB, such as dimethyl fumarate, curcumin, iguratimod, epigalocathechin gallate (EGCG), and DHMEQ inhibits ILPS-induced NF-κB activation and LPS-induced secretion of IL-1β (McGuire et al., 2016; Mucke, 2012; Peng et al., 2012; Suzuki and Umezawa, 2006; Wang et al., 2020; Wang et al., 2018; Wheeler et al., 2004).

TAK-242 (Matsunaga et al., 2011) inhibit TLR4 itself. There are several IRAK4 inhibitors (Lee et al., 2017). These molecules block the upstream signal to NF-κB activation. IRAK4 has recently attracted attention as a therapeutic target for inflammation and tumor diseases (Chaudhary et al., 2015).

LPS treatment induced a significant upregulation of the mRNA and release of IL-1β from retinal microglia. Minocycline inhibited its releases. Thus, minocycline might exert its antiinflammatory effect on microglia by inhibiting the expression and release of IL-1β (Wang et al., 2005).

Caspase-1 inhibition reduced the release of IL-1β in organotypic slices exposed to LPS+ATP. Administration of pralnacasan (intracerebroventricular, 50 μg) or belnacasan (intraperitoneal, 25–200 mg/kg) to rats blocked seizure-induced production of IL-1β in the hippocampus, and resulted in a twofold delay in seizure onset and 50% reduction in seizure duration (Ravizza et al., 2006).

Belnacasan, an orally active IL-1β converting enzyme/caspase-1 inhibitor, blocked IL-1β secretion with equal potency in LPS-stimulated cells from familial cold urticarial associated symdrome and control subjects (Stack et al., 2005).

In LPS-induced acute lung injury (ALI) mice model, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were

significantly decreased by cinnamaldehyde (CA) (Huang and Wang, 2017).

The suppressing capacities of six cinnamaldehyde-related compounds were evaluated and compared by using the LPS-primed and ATP-activated macrophages. At concentrations of 25~100 mM, cinnamaldehyde and 2-methoxy cinnamaldehyde dose-dependently inhibited IL-1β secretion (Ho et al., 2018).

In vitro, CA decreased the levels of pro-IL-1β and IL-1β in cell culture supernatants, as well as the expression of NLRP3 and IL-1β mRNA in cells. In vivo, CA decreased IL-1β production in serum. Furthermore, CA suppressed LPS-induced NLRP3, p20, Pro-IL-1β, P2X7 receptor (P2X7R) and cathepsin B protein expression in lung, as well as the expression of NLRP3 and IL-1β mRNA (Xu et al., 2017).

IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (anakinra) is fully active in blocking the IL-1R1, and therefore, the biological activities of IL-1α and IL-1β. The binding of IL-1α and IL-1β to IL-1R1 can be suppressed by soluble IL-1R like rilonacept (Kapur and Bonk, 2009). The binding of IL-1β to IL-1R1 can be inhibited by anti-IL-1β antibody (canakinumab and gevokizumab) (Church and McDermott, 2009) (Roell et al., 2010).

IL-1 is known to mediates autoinflammatory syndrome, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever. Blocking of binding of IL-1 to IL-1R1 by anakinra, canakinumab, and rilonacept have been already used to treat these autoinflammatory syndrome associated with overactivation of IL-1 signaling (Quartier, 2011).

Dex inhibits IL-1β gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-κB/Rel and AP-1 activation (Jeon et al., 2000).

Inhibition of IL-1 binding to IL-1R or the decreased production of IL-1b leads to the suppression of IL-1R signaling leading to NF-κB activation.

### Key Event Description

The NF-κB pathway consists of a series of events including IRAK (IL-1 receptor-associated kinase signaling), where the transcription factors of the NF-κB family play the key role. The canonical NF-κB pathway can be activated by a range of stimuli, including TNF receptor activation by TNF-a. Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates IkBa. This NF-κB inhibitor will be K48-linked ubiquitinated and degradated, allowing NF-κB to translocate to the nucleus. There, this transcription factor can express pro-inflammatory and anti-apoptotic genes. Furthermore, negative feedback genes are also transcribed and include IkBa and A20. When the NF-κB pathway is inhibited, its translocation will be delayed (or absent), resulting in less or no regulation of NF-κB target genes. This can be achieved by IKK inhibitors, proteasome inhibitors, nuclear translocation inhibitors or DNA-binding inhibitors (Gupta et al., 2010; Liu et al., 2017). Therefore, inhibition of IL-1R activation suppresses NF-κB.

In addition to the NF-kB pathway, IRAK activates a variety of transcription factors, including Interferon regulatory factor 5 (IRF5), Adaptor protein-1 (AP-1) and cAMP response element binding protein (CREB), resulting in the expression of broad array of inflammatory molecules and apoptosis-related proteins (Jain, 2014).

### How it is Measured or Detected

NF-κB transcriptional activity: Beta lactamase reporter gene assay (Miller et al. 2010)

NF-κB transcription: Lentiviral NF-κBGFP reporter with flow cytometry (Moujalled et al. 2012)

IκBα phosphorylation: Western blotting (Miller et al. 2010)

NF-κB p65 (Total/Phospho) ELISA

ELISA for IL-6, IL-8, and Cox

### References

Chaudhary, D., Robinson, S., Romero, D.L. (2015), Recent advances in the discovery of small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) as a therapeutic target for inflammation and oncology disorders. *J Med Chem* 58: 96-110, 10.1021/jm5016044

Church, L.D., McDermott, M.F. (2009), Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther* 11: 81-89,

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., et al. (1991), Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266: 10331-10336,

Gupta, S.C., Sundaram, C., Reuter, S., et al. (2010), Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy.

*Biochim Biophys Acta* 1799: 775-787, 10.1016/j.bbagrm.2010.05.004

Ho, S.C., Chang, Y.H., Chang, K.S. (2018), Structural Moieties Required for Cinnamaldehyde-Related Compounds to Inhibit Canonical IL-1beta Secretion. *Molecules* 23, 10.3390/molecules23123241

Huang, H., Wang, Y. (2017), The protective effect of cinnamaldehyde on lipopolysaccharide induced acute lung injury in mice. *Cell Mol Biol (Noisy-le-grand)* 63: 58-63, 10.14715/cmb/2017.63.8.13

Jain, A., Kaczanowska, S., Davila, E. (2014), IL-1 receptor-associated kinase signaling and its role in inflammation, cancer, progression, and therapy resistance. *Frontiers in Immunology* 5:553.

Jeon, Y.J., Han, S.H., Lee, Y.W., et al. (2000), Dexamethasone inhibits IL-1 beta gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-kappa B/Rel and AP-1 activation. *Immunopharmacology* 48: 173-183,

Kapur, S., Bonk, M.E. (2009), Rilonacept (arcalyst), an interleukin-1 trap for the treatment of cryopyrin-associated periodic syndromes. *P t* 34: 138-141,

Klein, S.L., Flanagan, K.L. (2016), Sex differences in immune responses. *Nat Rev Immunol* 16: 626-638, 10.1038/nri.2016.90

Lee, K.L., Ambler, C.M., Anderson, D.R., et al. (2017), Discovery of Clinical Candidate 1-{[(2S,3S,4S)-3-Ethyl-4-fluoro-5-oxopyrrolidin-2-yl]methoxy}-7-methoxyisoquinoli ne-6-carboxamide (PF-06650833), a Potent, Selective Inhibitor of Interleukin-1 Receptor Associated Kinase 4 (IRAK4), by Fragment-Based Drug Design. *J Med Chem* 60: 5521-5542, 10.1021/acs.jmedchem.7b00231

Liu, T., Zhang, L., Joo, D., et al. (2017), NF-kappaB signaling in inflammation. *Signal Transduct Target Ther* 2, 10.1038/sigtrans.2017.23

Matsunaga, N., Tsuchimori, N., Matsumoto, T., et al. (2011), TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. *Mol Pharmacol* 79: 34-41, 10.1124/mol.110.068064

McGuire, V.A., Ruiz-Zorrilla Diez, T., Emmerich, C.H., et al. (2016), Dimethyl fumarate blocks pro-inflammatory cytokine production via inhibition of TLR induced M1 and K63 ubiquitin chain formation. *Sci Rep* 6: 31159, 10.1038/srep31159

McKay, L.I., Cidlowski, J.A. (1999), Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 20: 435-459, 10.1210/edrv.20.4.0375

Mucke, H.A. (2012), Iguratimod: a new disease-modifying antirheumatic drug. *Drugs Today (Barc)* 48: 577-586, 10.1358/dot.2012.48.9.1855758

Peng, H., Guerau-de-Arellano, M., Mehta, V.B., et al. (2012), Dimethyl fumarate inhibits dendritic cell maturation via nuclear factor kappaB (NF-kappaB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. *J Biol Chem* 287: 28017-28026, 10.1074/jbc.M112.383380

Pernis, A.B. (2007), Estrogen and CD4+ T cells. *Curr Opin Rheumatol* 19: 414-420, 10.1097/BOR.0b013e328277ef2a

Quartier, P. (2011), Interleukin-1 antagonists in the treatment of autoinflammatory syndromes, including cryopyrin-associated periodic syndrome. *Open Access Rheumatol* 3: 9-18, 10.2147/oarrr.S6696

Ravizza, T., Lucas, S.M., Balosso, S., et al. (2006), Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. *Epilepsia* 47: 1160-1168, 10.1111/j.1528-1167.2006.00590.x

Roell, M.K., Issafras, H., Bauer, R.J., et al. (2010), Kinetic approach to pathway attenuation using XOMA 052, a regulatory therapeutic antibody that modulates interleukin-1beta activity. *J Biol Chem* 285: 20607-20614, 10.1074/jbc.M110.115790

Stack, J.H., Beaumont, K., Larsen, P.D., et al. (2005), IL-converting enzyme/caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. *J Immunol* 175: 2630-2634,

Straub, R.H. (2007), The complex role of estrogens in inflammation. *Endocr Rev* 28: 521-574, 10.1210/er.2007-0001

Suzuki, E., Umezawa, K. (2006), Inhibition of macrophage activation and phagocytosis by a novel NF-kappaB inhibitor, dehydroxymethylepoxyquinomicin. *Biomed Pharmacother* 60: 578-586, 10.1016/j.biopha.2006.07.089

Wang, A.L., Yu, A.C., Lau, L.T., et al. (2005), Minocycline inhibits LPS-induced retinal microglia activation. *Neurochem Int* 47: 152-158, 10.1016/j.neuint.2005.04.018

Wang, F., Han, Y., Xi, S., et al. (2020), Catechins reduce inflammation in lipopolysaccharide-stimulated dental pulp cells by inhibiting activation of the NF-kappaB pathway. *Oral Dis* 26: 815-821, 10.1111/odi.13290

Wang, Y., Tang, Q., Duan, P., et al. (2018), Curcumin as a therapeutic agent for blocking NF-kappaB activation in ulcerative colitis. *Immunopharmacol Immunotoxicol* 40: 476-482, 10.1080/08923973.2018.1469145

Wheeler, D.S., Catravas, J.D., Odoms, K., et al. (2004), Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1

beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. *J Nutr* 134: 1039-1044, 10.1093/jn/134.5.1039

Xu, F., Wang, F., Wen, T., et al. (2017), Inhibition of NLRP3 inflammasome: a new protective mechanism of cinnamaldehyde in endotoxin poisoning of mice. *Immunopharmacol Immunotoxicol* 39: 296-304, 10.1080/08923973.2017.1355377

## Event: 1702: Suppression of T cell activation

### Short Name: Suppression of T cell activation

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:277 - Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | KeyEvent |

### Biological Context

| Level of Biological Organization |
|---|
| Cellular |

### Cell term

| Cell term |
|---|
| T cell |

### Organ term

| Organ term |
|---|
| immune system |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

### Key Event Description

T cells are key orchestrators of the response against pathogens and are also fundamental in maintaining self-tolerance. A number of clinically important conditions have been described in which T-cell functions are altered, as in AIDS or upon immunosuppression after application of various immunosuppressive drugs to treat autoimmune disorders or allogeneic graft rejection. T-cell progenitors differentiate in the thymus into immature T cells that acquire the expression of the T-cell receptor (TCR), which recognizes antigen peptides from pathogens presented along with major histocompatibility complex (MHC). In addition to the TCR, T cells are

characterized by expression of the co-receptor molecules CD4 and CD8 on their cell surface. CD4+ T cells, also called T helper (Th) cells, recognize antigen/MHC-II complexes on antigen presenting cells (APCs) and coordinate the activation of other immune cells including B cells, macrophages, etc.

Therefore, CD4+ T cells are crucial for coordination of the immune response and for the elimination of invading pathogens. On the other hand, CD8+ T cells, referred to as T cytotoxic cells, recognize antigen/MHC-I complexes and are responsible for the killing of pathogen-infected cells.

T-cell activation and differentiation depends on antigen presenting cells (APCs) such as dendritic cells (DCs), macrophages and B cells. Depending on the insult affecting a given tissue, . Different subsets of DCs can be generated that in turn are able to coordinate the differentiation of a particular Th subset. To date, the following Th subsets have been described: Th1, Th2, Th9, Th17, Th22, Tfh (follicular helper T cells), Tr1 (type 1regulatory T cells) and Treg (regulatory T cells), each possessing a specific function in the elimination of pathogens. (reviewed by Simeoni et al. (Simeoni et al., 2016))

Although CD4 T cells are able to commit to Th1, Th2 and Th17 lineages in the absence of IL-1R signaling at steady state, these committed CD4 T cells are unable to effectively secrete their cytokines upon TCR ligation. Namely, IL-1 is indispensable for CD4 T cell effector function. (Lin et al, 2015)

Moreover, since full activation of B cells and antibody production and class switch depends on T cell help. The impaired activation of T cells leads to impaired B cell activation and antibody production (reviewed by Mok (Mok, 2010)).

### How it is Measured or Detected

T cell activation can be evaluated by measuring IL-2 production by ELISA or T cell proliferation by incorporation of the analysis of CFSE labeled T cells or [$^{3}$H]thymidine incorporation.

### References

Lin, D., Lei, L., Zhang, Y., et al. (2015), Secreted IL-1alpha promotes T-cell activation and expansion of CD11b(+) Gr1(+) cells in carbon tetrachloride-induced liver injury in mice. *Eur J Immunol* 45: 2084-2098, 10.1002/eji.201445195

Mok, M.Y. (2010), The immunological basis of B-cell therapy in systemic lupus erythematosus. *Int J Rheum Dis* 13: 3-11, 10.1111/j.1756-185X.2009.01458.x

Simeoni, L., Thurm, C., Kritikos, A., et al. (2016), Redox homeostasis, T cells and kidney diseases: three faces in the dark. *Clin Kidney J* 9: 1-10, 10.1093/ckj/sfv135

Weih, F., Carrasco, D., Durham, S.K., et al. (1995), Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. *Cell* 80: 331-340,

## List of Adverse Outcomes in this AOP

**Event: 986: Impairment of TDAR**

### Short Name: Impairment, Impairment of TDAR

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:277 - Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | AdverseOutcome |

### Stressors

| Name |
|---|
| IL-1 receptor antagonist IL-1Ra (Anakinra) |
| anti-IL-1b antibody (Canakinumab) |
| soluble IL-1R (Rilonacept) |

### Biological Context

**Level of Biological Organization**

Individual

**Level of Biological Organization**

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

CNIs induced impairment of TDAR is demonstrated with rodent studies. That is, oral administration of FK506 or CsA to mice for 4 days impaired the response of PFC in splenocytes after intravenous immunization with sheep erythrocytes (Kino et al. 1987). Likewise, oral administration of FK506 to rats over a four-week period reduced production of both anti-KLH-IgG and IgM antibodies after subcutaneous immunization with KLH (Ulrich et al. 2004). Moreover, Treatment with CsA at 50 mg/kg BID via oral gavage in cynomolgus monkey resulted in reduction of serum SRBC-specific IgM and IgG (Kevin, G. et al. 2014). As for humans, in vitro experiments showed that treatment with FK506 or CsA of peripheral blood mononuclear cells from blood-bank donors suppressed the production of IgM and IgG antibodies specific to T-cell–dependent antigens. (Heidt et al, 2009) Also, in SKW6.4 cells (IL-6–dependent, IgM-secreting, human B-cell line) cultures, FK506 or CsA suppressed the production of IgM antibodies in the presence of T-cell activation. (Sakuma et al. 2001b) Considering that FKF506 and CsA reduce T cell-derived cytokines including IL-2 and IL-4, these findings strongly suggest that impairment of TDAR following reduced production of such cytokines occurs at least in common among humans, monkey and rodents.

## Key Event Description

Antibody production to T-cell–dependent antigens is established through the coordination of B cells, antigen-presenting cells as well as T-cell–derived cytokines, which stimulate B cells to proliferate and differentiate. T-cell–dependent antibody response (TDAR) might be altered if any of these cell populations is affected.

Interleukin (IL)-2 stimulates B cells to proliferate through surface IL-2 receptors. IL-4 stimulates B-cells to proliferate, to switch immunoglobulin classes, and to differentiate into plasma and memory cells. Suppressing the production of these B-cell–related cytokines appear to be impaired TDAR, as seen in the result of FK506 treatment (Heidt et al, 2009).

IL-2 and IL-4 are produced and secreted by helper T cells and play important roles in the development of TDAR. IL-4 affects maturation and class switching of B cells as well as proliferation, both of which induces/enhances T cell dependent antibody production. IL-2 promotes differentiation of B cells through IL-2 stimulates differentiation of the activated T cell into T cell called Th2 cell. Therefore, suppressed production of IL-2 and IL-4 impairs TDAR (Alberts et al. 2008).

In male CD-1 mice, chronic psychosocial stress (types of social outcome occurred: residents becoming subordinates) decrease in anti- keyhole limpet hemocyanine (KLH) immunoglobulin (Ig)G. (Alessandro, B. et al. 2003).

In female B6C3F1 mice, 1,2:5,6-dibenzanthracene (DBA) exposure reduced total IgG antibody in spleen cell culture supernatants after in vitro stimulation with lipopolysaccharide (LPS) (Donna, C. et al. 2010).

Treatment with cyclosporin A (CsA) at 50 mg/kg BID via oral gavage in cynomolgus monkey resulted in reduction of serum sheep red blood cells (SRBC)-specific IgM and IgG (Kevin, G. et al. 2014).

After a 9-day culture of B cells and non-pre-activated T cell stimulation with FK506 or CsA, the levels of IgM and IgG in the culture supernatant were reduced at 0.3 and 1.0 ng/mL (0.37 and 1.24 nM) of FK506 or 50 and 100 ng/mL (41.6 and 83.2 nM) of CsA (Heidt et al, 2009).

After a 4-day culture of SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) and anti-CD3/CD28 stimulated peripheral blood mononuclear cells (PBMC) culture supernatant with FK506 or CsA, the level of IgM in the culture supernatant was reduced at concentrations of 0.01 to 100 ng/mL (0.012 to 124 nM) of FK506 or 0.1 to 1000 ng/mL (0.083 to 83.2 nM) of CsA (Sakuma et al. 2001b).

Rats were treated with FK506 for over four weeks and immunized with KLH, after which serum concentration of anti-KLH IgM and IgG was reduced at the dose level of 3 mg/kg/day (Ulrich et al. 2004).

Mice were treated with FK506 or CsA for 4 days, and immunized with SRBC, after which antigen-specific plaque-forming splenocytes were reduced at dose levels of 3.2, 10, 32 and 100 mg/kg of FK506 or 32 and 100 mg/kg of CsA (Kino et al. 1987b).

As immunosuppression-derived adverse outcomes by calcineurin inhibition, FK506 and CsA increase the frequency and/or severity of infections and allergic reactions impaired TDAR deems to be one of the causative factors for these side effects. Some clinical trials of FK506 and CsA revealed these adverse effects as follows.

- In clinical trials of renal transplantation using FK506 or CsA, opportunistic infections such as candida, cytomegalovirus and herpes simplex virus were reported (Ekberg et al. 2007).
- In recipients of liver transplants treated with FK506 or CsA, opportunistic infections such as cytomegalovirus, hepatitis C virus, hepatitis B and herpes simplex virus were reported (Fung et al. 1991).
- Cardiac transplant patients treated with cyclosporin developed pulmonary infections within the first year after surgery (Luster, M.I. et al. 1993).
- In patients of X-linked autoimmune enteropathy treated with CsA or FK506, serum levels of IgE developed extremely high during the immunosuppressive therapy (Kawamura et al. 1997).
- Renal transplant recipients treated with belatacepy/mycophenolate (MMF)/predonisone or FK506/MMF/prednisone showed significantly lower the geometric mean hemagglutination inhibition titer against influenza vaccine, hemagglutination-specific IgG and isotype IgG1 antibodies, and IgG-antibody secreting cells response (Gangappa et al. 2019).

### How it is Measured or Detected

TDAR could be examined in vivo and in vitro. In vivo studies of antigen-specific antibodies are usually performed by measuring serum antibody levels with Enzyme-Linked ImmunoSorbent Assay (ELISA) or with a plaque-forming cell (PFC) assay.

- Rats were repeatedly administered FK506 orally for 4 weeks and immunized with KLH, after which the serum was examined for T-cell–dependent, antigen-specific, IgM and IgG levels using a Sandwich ELISA kit (Ulrich et al. 2004).
- Mice were repeatedly administered calcineurin inhibitors (CNIs) including FK506 and CsA orally for 4 days and immunized with SRBC, after which spleen cells were examined using a PFC assay (Kino et al. 1987).
- Cynomolgus monkeys received 50 mg/kg CsA twice a day via oral gavage (10 h apart) for 23 days and were immunized with SRBC, after which the serum was examined for Anti-SRBC IgM and IgG levels using an ELISA specific for SRBC antigen (Kevin, G. et al. 2014).
- Mice were exposed a single pharyngeal aspiration of DBA, after which supernatants of splenocytes cultured for 24 h in the presence of LPS and assayed using a mouse IgM or IgG matched pairs antibody kit (Bethyl Laboratories, Montgomery, TX) (Donna, C. et al. 2010).

For in vitro studies, total IgM and IgG levels in culture supernatant are often measured after polyclonal T-cell activation rather than measuring antigen stimulation in immune cell cultures.

- T cells and B cells isolated from human peripheral blood mononuclear cells (PBMC) were co-cultured with a CNIs for nine days in the presence of polyclonal–T-cell stimulation, after which supernatants were tested for immunoglobulin IgM and IgG levels using a Sandwich ELISA kit. Treatment with FK506 or CsA reduced the levels of IgM and IgG at the concentrations of 0.3 and 1.0 ng/mL or 50 and 100 ng/mL (Heidt et al, 2009).
- SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) were cultured with anti-CD3/CD28 antibody-stimulated PBMC culture supernatant. After culturing for four days, IgM produced in the culture supernatants was measured using an ELISA kit. FK506 or CsA reduced the levels of IgM at the concentrations of 0.01 to 100 ng/mL or 0.1 to 1000 ng/mL (Sakuma et al.

2001b).
- In order to examine class switching, T cells derived from human PBMCs were cultured with CNIs, and cytokine mRNA levels of Interferon-gamma, IL-2, IL-4, IL-5, IL-10, IL-13, and other B-cell–stimulatory cytokines produced in T cells were measured by quantitative PCR (Dumont et al. 1998).

### Regulatory Significance of the AO

The ICH S8 guideline, which covers immunosuppression of small molecule drugs, determines the need for immunotoxicity studies by comprehensively evaluating the findings of pharmacology, changes in the immune system in repeated-dose toxicity studies, and other factors using a Weight of Evidence approach. If there is concern about immunotoxicity, the presence or absence of immunotoxicity should be determined using an in vivo test system capable of assessing the functional changes of predicted immunotoxic target cells. If immunotoxicity is observed, additional studies including in vitro assays or clinical evaluation should be considered to assess the risk of immunotoxicity in humans. Because TDAR involves many immune cell populations, including T cells, B cells, and antigen-presenting cells, evaluation of TDAR is recommended when there is concern about immunotoxicity but the immunotoxic target cells are unclear. The S8 guidelines list KLH, SRBC, and tetanus toxin as antigens for TDAR.

The draft FDA immunotoxicity testing guidance (2020) covers immunosuppressive and immunostimulatory drugs and biologics; evaluating immunosuppressive drugs in the draft FDA guidance is similar to that in the S8 guideline, with in vivo TDAR assays recommended when toxic target cells are unknown. The draft guidance states that TDAR assays using KLH as an antigen have been established in mice, rats, dogs, minipigs, and cynomolgus monkeys, but the use of SRBC and tetanus toxin as antigens is also acceptable.

For the assessment for pesticides, US EPA OPPTS 870.7800 immunotoxicity testing guideline recommends TDAR using SRBC. The REACH guideline does not provide for immunotoxicity testing, but it provides triggers for conducting immunotoxicity testing.

The WHO/IPSS Immunotoxicity Risk assessment Guidance (2012) describes a strategy for assessing five categories of immunotoxicity risks, including immunosuppression. For risk assessment of immunosuppression, it calls for identification of immunosuppression risks, prediction of pathogenesis that may occur, and consideration of safety margins based on the WoE approach from human findings, infection resistance tests, immune function tests, general immune system assays, histopathological findings and organ weights in general toxicity studies, and hematological data.

The evaluation of immunotoxicity in F1 animals in the OECD Guidelines for Extended First Generation Reproductive and Developmental Toxicity Studies (TG443) requires that PFC and ELSA assays to measure primary IgM antibody production by TDAR using T-cell dependent antigens (SRBC, KLH, etc.) be performed. Furthermore, if changes are observed, the significance of the changes should be examined by comprehensively evaluating other data.

The outcomes of immunosuppression are susceptibility to infection and tumorigenesis, and the FDA guidance requires that immunosuppressive drugs be evaluated for carcinogenic risk using WoE approach based on the results of carcinogenicity and immunotoxicity studies. Meanwhile, the ICH S1B(R1) Draft Step 2 Guidelines for Carcinogenicity Testing calls for evaluation of carcinogenicity by WoE approach instead of rat carcinogenicity testing, because rodent carcinogenicity test models are less capable of detecting carcinogenicity. On the other hand, it is difficult to define susceptibility to infection as a measurable AO with a clear mechanism, because immune responses vary among pathogens. In fact, many immunotoxicity guidelines require that the risk of immunotoxicity be identified and assessed by evaluating immune functions.

In AOP277, it was difficult to define susceptibility to infection as an AO for the AOP154, so TDAR, which is recommended as an indicator of immunosuppresoin in many guidelines, was used as an AO. It is expected that several AOPs with TDARs as AOs will be developed, and based on these AOPs, it may be possible to develop an IATA to assess the risk of immunotoxicity characterized by TDARs.

### References

Alberts, B., Johnson, A., Lewis, L., Raff, M., Roberts, K. and Walter, P. (2008). Molecular Biology of the Cell. 5th ed., Garland Science, New York. 1539-1601

Heidt, S., Roelen, D. L., Eijsink, C., Eikmans, M., van Kooten, C., Claas, F. H. and Mulder, A. (2010). Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. Clinical and experimental immunology. 159(2): 199-207.

Sakuma, S., Kato, Y., Nishigaki, F., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2001b). Effects of FK506 and other

immunosuppressive anti-rheumatic agents on T cell activation mediated IL-6 and IgM production in vitro. International Immunopharmacology 1(4): 749-57.

Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Imanaka, H. (1987). FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. Journal of antibiotics. 40(9): 1249-1255.

Ulrich, P., Paul, G., Perentes, E., Mahl, A., and Roman D. (2004). Validation of immune function testing during a 4-week oral toxicity study with FK506. Toxicology Letters 149(1-3): 123-31.

Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.

Ekberg, H., Tedesco-Silva, H., Demirbas, A., Vítko, S., Nashan, B., Gürkan, A., Margreiter, R., Hugo, C., Grinyó, J.M., Frei, U., Vanrenterghem, Y., Daloze, P. and Halloran, P.F.; ELITE-Symphony Study. (2007). Reduced exposure to calcineurin inhibitors in renal transplantation. The New England journal of medicine 357 (25): 2562-75.

Fung, J., Abu-Elmagd, K., Jain, A., Gordon, R., Tzakis, A., Todo, S., Takaya, S., Alessiani, M., Demetris, A., Bronster, O., Martin, M., Mieles, L., Selby, R., Reyes, J., Doyle, H., Stieber, A., Casavilla, A. and Starzl, T. (1991). A randomized trial of primary liver transplantation under immunosuppression with FK 506 vs cyclosporine. Transplantation proceedings 23 (6): 2977-83.

Luster, M.I., and Rosenthal, G.J. (1993). Environmental Health Perspectives. 100: 219-36.

Alessandro B, Paola S, Alberto E. Paneraic, Tiziana P,Paola Palanzaa and Stefano P(2003). Chronic psychosocial stress-induced down-regulation of immunity depends upon individual factors Journal of Neuroimmunology 141: 58–64

Donna C. S, Matthew J. S and Kimber L. W Jr. (2010) Systemic immunosuppression following a single pharyngeal aspiration of 1,2:5,6-dibenzanthracene in female B6C3F1 mice, Journal of Immunotoxicology, 7:3, 219-231

Kevin G, Hossein S, Raju S, Valerie A, Anna K, Ming Z, Fen-Fen L, Hung Q. N, Lei Z, John K. S, Min W and Helen J. M(2015) Inhibition of CRAC with a human anti-ORAI1 monoclonal antibody inhibits T-cell-derived cytokine production but fails to inhibit a T-cell-dependent antibody response in the cynomolgus monkey, Journal of Immunotoxicology, 12:2, 164-173,

Gangappa S, Wrammert J, Wang D, Li ZN, Liepkalns JS, Cao W, Chen J, Levine MZ, Stevens J, Sambhara S, Begley B, Mehta A, Pearson TC, Ahmed R, Larsen CP. (2019) Kinetics of antibody response to influenza vaccination in renal transplant recipients. Transpl Immunol. 53:51-60.

Kawamura N, Furuta H, Tame A, Kobayashi I, Ariga T, Okano M, Sakiyama Y. (1997) Extremely high serum level of IgE during immunosuppressive therapy: paradoxical effect of cyclosporine A and tacrolimus. Int Arch Allergy Immunol. 112(4):422-4.

# Appendix 2

## List of Key Event Relationships in the AOP

### List of Adjacent Key Event Relationships

**Relationship: 2002: Impaired IL-1R1 signaling leads to Inhibition, Nuclear factor kappa B (NF-kB)**

#### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | adjacent | High | Moderate |

#### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

## Key Event Relationship Description

After binding of IL-1  or IL-1  to IL-1R, IL-1 and IL-1R1 facilitates recruitment of IL-1RacP. Then this trimeric complex rapidly assembles two intracellular signaling proteins, myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor–activated protein kinase (IRAK) 4. IL-1, IL-1RI, IL-RAcP, MYD88, and IRAK4 form a stable IL-1–induced first signaling module. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB.

## Evidence Supporting this KER

Mice lacking MYD88 or IRAK4 show severe defects in IL-1 signaling (Adachi et al., 1998; Suzuki et al., 2002). In the cell culture, lacking MYD88 show a block of NF-κB activation by IL-1 (Medzhitov et al., 1998). MyD88 can strongly activate an AP-1 and this activity is inhibited by dominant-negative TRAF6; therefore, MyD88 and TRAF6 are involved in IL-1R-mediated NF-κB activation, and both activate AP-1 (Medzhitov et al., 1998). Similarly, humans with mutations in the IRAK4 gene have defects in IL-1RI and Toll-like receptor (TLR) signaling (Picard et al., 2003).

**Biological Plausibility**

The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1RI that facilitates recruitment of IL-1RacP (Cavalli et al., 2015). Through conserved cytosolic regions called Toll- and IL-1R–like (TIR) domains (Radons et al., 2003), the trimeric complex rapidly assembles two intracellular signaling proteins, myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor–activated protein kinase (IRAK) 4 (Brikos et al., 2007; Li et al., 2002). IL-1, IL-1RI, IL-RAcP, MYD88, and IRAK4 form a stable IL-1–induced first signaling module. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB reviewed by (Brikos et al., 2007; Weber, Wasiliew and Kracht, 2010).

**Empirical Evidence**

**IL-1Ra blocks IL-1 signaling:**

IL-1Ra down modulation of EGF receptor (3 nM of ED50) (Dripps et al., 1991)

IL-1Ra suppression of IL-1-induced endothelial cell-leukocyte adhesion (approximately 10 ng/ml of ED50) (Dripps et al., 1991)

IL-1Ra suppresses rhIL-1a-induced mouse thymocytes proliferation (ED50 almost 3 mg/mL) (Arend et al., 1990)

IL-1Ra competed for binding of $^{125}$I-IL-1a to type I IL-1R present on EL4 thymoma cells, 3T3 fibroblasts, hepatocytes, and Chinese hamster ovary cells expressing recombinant mouse type I IL-1R. The IC50 values for IL-1ra binding (ranging from 2 to 4 ng/ml) were similar to those of IL-1a. (McIntyre et al., 1991)

Recombinant mIL-1Ra competitively inhibited $^{125}$I-labeled IL-1 alpha binding to murine type I IL-1R present on EL4 6.1 cells (Ki value of 0.21 nM) and antagonized IL-1-stimulated co-mitogenesis in murine thymocytes (0.7 x 10(6)-1.1 x 10(6) units/mg). (Shuck et al., 1991)

Peripheral blood mononuclear cells (PBMC) obtained after completion of the IL-Ira infusion synthesized significantly less interleukin 6 ex vivo than PBMC from saline-injected controls. (Granowitz et al., 1992)

**Canakinumab (ACZ885, Ilaris):**

Canakinumab binds to human IL-1β with high affinity; the antibody-antigen dissociation equilibrium constant is approximately 35–40 pM(Dhimolea, 2010).

The antibody binds to human IL-1β with high affinity (about 40 pmol/l). The antibody was found to neutralize the bioactivity of human IL-1β on primary human fibroblasts in vitro 44.6 pmol/l (7.1 ± 0.56 ng/ml; n = 6) of ED50. Application of Canakinumab

intraperitoneally 2 hours before injecting the IL-1β producing cells completely suppressed joint swelling in mouse models of arthritis (0.06 mg/kg of EC50) (Alten et al., 2008).

Primary human fibroblasts are stimulated with recombinant IL-1b or conditioned medium obtained from LPS-stimulated human PBMCs in the presence of various concentrations of Canakinumab or IL-1RA ranging from 6 to 18,000 pM. Supernatant is taken after 16 h stimulation and assayed for IL-6 by ELISA. Canakinumab typically have 1 nM or less of EC50 for inhibition of IL-6 production (Canakinumab Patent Application WO02/16436.)

**Rilonacept (IL-1 Trap, Arcalyst):**

Incubation of the human MRC5 fibroblastic cell line with IL-1β induces secretion of IL-6. At a constant amount of IL-1β (4 pM), the IC50 of the IL-1 trap is ~2 pM. Another unique property of the IL-1 trap is that it not only blocks IL-1β, but also blocks IL-1α with high affinity (KD = ~3 pM; data not shown). The titration curve of IL-1 trap in the presence of 10 pM IL-1β shows an IC50 of 6.5 pM, which corresponds to a calculated KD of 1.5 pM (This affinity is 100 times higher than that of the soluble single component receptor IL-1RI (Economides et al., 2003).

**IRAK4 inhibitor:**

By reconstituting IRAK-4-deficient cells with wild type or kinase-inactive IRAK-4, it is demonstrated that the kinase activity of IRAK-4 is required for the optimal transduction of IL-1-induced signals, including the activation of IRAK-1, NF-κB, and JNK, and the maximal induction of inflammatory cytokines (Lye et al., 2008).

Various concentrations of kinase-active or kinase-inactive IRAK-4 were transiently overexpressed in IRAK-4-deficient cells that were also transiently transfected with an NF-κB-dependent luciferase reporter and α-galactosidase expression vector. IRAK-4 is recruited to the IL-1R-associated complex 1 min after IL-1β treatment (10 ng/mL). Transfected cells were left untreated or treated with IL-1β (10 ng/ml) for 6 h before luciferase and α-galactosidase activities were measured. The luciferase activity was divided by the α-galactosidase activity, and fold activation was calculated compared with the activity of untreated cells carrying an empty α-vector (normalized as 1). The results demonstrated that kinase-active IRAK-4 dose dependently activates IL-1-mediated NF-κB. Kinase-inactive IRAK-4 expression resulted in severely reduced IL-1 responses and defective NF-κB and JNK activation induced by IL-1 (Lye et al., 2004).

## Quantitative Understanding of the Linkage

See Empirical Evidence.

**Response-response relationship**

**IL-1Ra blocks IL-1 signaling:**

Suppression of IL-1-induced IL-1, TNFa, or IL-6 synthesis was dose-dependent (P ≦ .0001). At a twofold molar excess, IL-Ira inhibited IL-1-induced IL-1 or TNFa synthesis by 50% (P < .01); an equimolar concentration of IL-Ira inhibited synthesis of these two cytokines by over 20% (P < .05). A 10-fold molar excess of IL-Ira over IL-Ib reduced IL-Ib-induced IL-Ia by 95% (P = .01) and IL-Ia-induced IL-1b by 73% (P < .01). In elutriated monocytes, a 10-fold molar excess of IL-Ira reduced IL-Ib-induced IL-Ia by 82% (P < .05), TNFa by 64% (P = .05), and IL-6 by 47% (P < .05). (Granowitz et al., 1992)

**Rilonacept (IL-1 Trap, Arcalyst) blocks IL-1 signaling:**

The titration curve of IL-1 trap in the presence of 10 pM IL-1β shows an IC50 of 6.5 pM, which corresponds to a calculated KD of 1.5 pM (This affinity is 100 times higher than that of the soluble single component receptor IL-1RI (Economides et al., 2003).

## References

Adachi, O., Kawai, T., Takeda, K., et al. (1998), Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 9: 143-150,

Alten, R., Gram, H., Joosten, L.A., et al. (2008), The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. *Arthritis Res Ther* 10: R67, 10.1186/ar2438

Arend, W.P., Welgus, H.G., Thompson, R.C., et al. (1990), Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest* 85: 1694-1697, 10.1172/jci114622

Dhimolea, E. (2010), Canakinumab. *MAbs* 2: 3-13,

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., et al. (1991), Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 266: 10331-10336,

Economides, A.N., Carpenter, L.R., Rudge, J.S., et al. (2003), Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat Med* 9: 47-52, 10.1038/nm811

Granowitz, E.V., Clark, B.D., Vannier, E., et al. (1992), Effect of interleukin-1 (IL-1) blockade on cytokine synthesis: I. IL-1 receptor

antagonist inhibits IL-1-induced cytokine synthesis and blocks the binding of IL-1 to its type II receptor on human monocytes. *Blood* 79: 2356-2363,

Lye, E., Dhanji, S., Calzascia, T., et al. (2008), IRAK-4 kinase activity is required for IRAK-4-dependent innate and adaptive immune responses. *Eur J Immunol* 38: 870-876, 10.1002/eji.200737429

Lye, E., Mirtsos, C., Suzuki, N., et al. (2004), The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. *J Biol Chem* 279: 40653-40658, 10.1074/jbc.M402666200

McIntyre, K.W., Stepan, G.J., Kolinsky, K.D., et al. (1991), Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. *J Exp Med* 173: 931-939,

Medzhitov, R., Preston-Hurlburt, P., Kopp, E., et al. (1998), MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 2: 253-258,

Picard, C., Puel, A., Bonnet, M., et al. (2003), Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 299: 2076-2079, 10.1126/science.1081902

Shuck, M.E., Eessalu, T.E., Tracey, D.E., et al. (1991), Cloning, heterologous expression and characterization of murine interleukin 1 receptor antagonist protein. *Eur J Immunol* 21: 2775-2780, 10.1002/eji.1830211119

Suzuki, N., Suzuki, S., Duncan, G.S., et al. (2002), Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature* 416: 750-756, 10.1038/nature736

## Relationship: 2003: Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | adjacent | High | Moderate |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

### Key Event Relationship Description

NF-kB plays a crucial role in the activation of dendritic cells as well as T cells. In dendritic cells, the activation of the canonical NF-kB pathway in response to pro-inflammatory stimuli, such as cytokines including IL-1a or IL-1b and TLR ligands, stimulate the maturation of dendritic cells with enhanced antigen presenting function. The inhibition of NF-kB suppress antigen presenting function of dendritic cells, resulting in suppression of T cell activation (reviewed by Reinhard et al (Reinhard et al., 2012) and van Delft et al (van Delft, Huitema and Tas, 2015).

In T cells, NF-kB can be activated by several pathways of signal transduction. The engagement of the TCR by major histocompatibility complex (MHC) plus antigen initiates downstream CD3 immunotyrosine activation motif (ITAM) phosphorylation by the Src family kinases, FYN and leukocyte C-terminal src kinase (LCK). Phosphorylated CD3 activates the T cell specific tyrosine kinase, zeta-chain associated protein kinase (ZAP-70), which ultimately trigger calcium release and protein kinase (PK)C activation, respectively. Activation of a specific PKC isoform, PKCμ, connects the above described TCR proximal signaling events to distal

events that ultimately lead to NF-kB activation. Importantly, PKCm activation is also driven by engagement of the T cell co-stimulatory receptor CD28 by B7 ligands on antigen presenting cells (APCs). In addition, the stimulation of T cells by IL-1 activates NF-kB as already described before. Once in the nucleus, NF-kB governs the transcription of numerous genes involved in T cell survival, proliferation, and effector functions (Paul and Schaefer, 2013).

## Evidence Supporting this KER

Mice lacking NF-kB p50 are unable to effectively clear L. monocytogenes and are more susceptible to infection with S. peumoniae (Sha et al., 1995).

**Biological Plausibility**

Although CD4 T cells are able to commit to Th1, Th2 and Th17 lineages in the absence of IL-1R signaling at steady state, these committed CD4 T cells are unable to effectively secrete their cytokines upon TCR ligation. Namely, IL-1 is indispensable for CD4 T cell effector function. (Lin et al, 2015)

RelB deficient mice had an impaired cellular immunity, as observed in contact sensitivity reaction (Weih et al., 1995).

Delayed-type hypersensitivity (DTH) responses were significantly suppressed in IL-1b-deficient and IL-1a/b-deficient mice. Lymph node cells derived from antigen-sensitized IL-1b-deficient and IL-1a/b-deficient mice and IL-1R type I-deficient mice, exhibited reduced proliferative responses against antigen. Antigen-specific CD4+ T cell proliferative responses were significantly reduced following co-culture with IL-1RI–/– dendritic cells (DCs) (Nambu et al., 2006).

**Empirical Evidence**

RelB deficient mice had an impaired cellular immunity, as observed in contact sensitivity reaction (Weih et al., 1995).

Quite a few NF-kB inhibitors have been reported. MG132, bortezomib, curcumin, DHMEQ(Dehydroxymethylepoxyquinomicin), naringin, sorafenib, genistein and parthenolide are some of representatives (Pordanjani and Hosseinimehr, 2016).

Interferon-γ (IFN-γ) production in response to CMV-infected fibroblasts was reduced under the influence of MG132, a proteosome inhibitor as well as a NF-kB inhibitor, in a dose-dependent manner. A marked reduction was observed at 0.5 μM. Likewise, CMV-specific cytotoxicity of CD8(+) T cells was decreased in the presence of MG132 (Wang et al., 2011).

In vivo MG132 administration to NC/Nga mice with DNFB-induced dermatitis reduced Th17 cells but maintained the level of Th1 cells, resulting in the alleviation of dermatitis lesions by decreasing both serum IgE hyperproduction and mast cell migration (Ohkusu-Tsukada et al., 2018).

Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both in vivo and in vitro (Satou et al., 2004). Bortezomib inhibits T-cell function versus infective antigenic stimuli in a dose-dependent manner in vitro (Orciuolo et al., 2007).

Dehydroxymethylepoxyquinomicin (DHMEQ), a novel nuclear factor-kappaB inhibitor, induces selective depletion of alloreactive or phytohaemagglutinin-stimulated peripheral blood mononuclear cells, decreases production of T helper type 1 cytokines, and blocks maturation of dendritic cells (Nishioka et al., 2008).

Regarding the suppression of NF-kB by impaired IL-1 signaling, it was reported that delayed-type hypersensitivity (DTH) responses were significantly suppressed in IL-1β-deficient and IL-1α/β-deficient mice. Lymph node cells derived from antigen-sensitized IL-1β-deficient and IL-1α/β-deficient mice and IL-1R type I-deficient mice, exhibited reduced proliferative responses against antigen. These data suggest that IL-1β is necessary for the efficient priming of T cells. In addition, CD4+ T cell-derived IL-1 plays an important role in the activation of DCs during the elicitation phase, resulting in the production of TNF, that activate allergen-specific T cells (Nambu et al., 2006).

## Quantitative Understanding of the Linkage

A representative NF-kB inhibitor, MG132 that suppresses NF-kB activity at more than 10 mM (Fiedler et al. 1998) suppresses IL-2-induced activation of STAT5 at 50 mM. (Yu and Malek., 2001). However, MG-132 did not decrease the effect of TNF-α-induced NF- κ B activation on AP-1 activation (Fiedler, Wernke-Dollries and Stark, 1998).

A representative NF-kB inhibitor, DHMEQ (1μg/mL) blocked phytohaemagglutinin (PHA-)-induced nuclear translocation of NF-kB in Jurkat cells via inhibition of degradation of IkBa. Preincubation of peripheral blood mononuclear cells and Jurkat cells with DHMEQ (1 μg/ml, 3 hr) greatly reduced PHA-stimulated expression of IFN- , IL-2 and TNF- genes although DHMEQ alone without PHA-stimulation did not affect cytokine production in unstimulated PBMC. DHMEQ (0·5–3 μg/mL, 3 days) inhibited PHA-stimulated proliferation of peripheral blood mononuclear cells (PBMC) in a dose-dependent manner although did not affect the viability of resting PBMC under identical culture conditions. DHMEQ (3 μg/mL, 24 hr) induced apoptosis of PHA-stimulated PBMC. DHMEQ (0·5 μg/mL) decreased levels of TNF-α-stimulated expression of CD40 in monocyte-derived dendritic cells (DCs). Exposure of DCs to DHMEQ (0·5 or 1 μg/ml) reduced their endocytic ability (Nishioka et al., 2008).

**Response-response relationship**

Interferon-γ (IFN-γ) production in response to CMV-infected fibroblasts was reduced under the influence of MG132 in a dose-dependent manner. A marked reduction was observed at 0.5 µM. Likewise, CMV-specific cytotoxicity of CD8(+) T cells was decreased in the presence of MG132 (Wang et al., 2011).

Bortezomib (1 mg/kg) inhibits T-cell function versus infective antigenic stimuli in vitro (Orciuolo et al., 2007).

### References


Fiedler, M.A., Wernke-Dollries, K., Stark, J.M. (1998), Inhibition of TNF-alpha-induced NF-kappaB activation and IL-8 release in A549 cells with the proteasome inhibitor MG-132. *Am J Respir Cell Mol Biol* 19: 259-268, 10.1165/ajrcmb.19.2.3149

Lin, D., Lei, L., Zhang, Y., et al. (2015), Secreted IL-1alpha promotes T-cell activation and expansion of CD11b(+) Gr1(+) cells in carbon tetrachloride-induced liver injury in mice. *Eur J Immunol* 45: 2084-2098, 10.1002/eji.201445195

Nambu, A., Nakae, S., Iwakura, Y. (2006), IL-1beta, but not IL-1alpha, is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses. *Int Immunol* 18: 701-712, 10.1093/intimm/dxl007

Nishioka, C., Ikezoe, T., Jing, Y., et al. (2008), DHMEQ, a novel nuclear factor-kappaB inhibitor, induces selective depletion of alloreactive or phytohaemagglutinin-stimulated peripheral blood mononuclear cells, decreases production of T helper type 1 cytokines, and blocks maturation of dendritic cells. *Immunology* 124: 198-205, 10.1111/j.1365-2567.2007.02755.x

Ohkusu-Tsukada, K., Ito, D., Takahashi, K. (2018), The Role of Proteasome Inhibitor MG132 in 2,4-Dinitrofluorobenzene-Induced Atopic Dermatitis in NC/Nga Mice. *Int Arch Allergy Immunol* 176: 91-100, 10.1159/000488155

Orciuolo, E., Galimberti, S., Petrini, M. (2007), Bortezomib inhibits T-cell function versus infective antigenic stimuli in a dose-dependent manner in vitro. *Leuk Res* 31: 1026-1027, 10.1016/j.leukres.2006.09.002

Reinhard, K., Huber, M., Lohoff, M., et al. (2012), The role of NF-kappaB activation during protection against Leishmania infection. *Int J Med Microbiol* 302: 230-235, 10.1016/j.ijmm.2012.07.006

Sha, W.C., Liou, H.C., Tuomanen, E.I., et al. (1995), Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 80: 321-330,

van Delft, M.A., Huitema, L.F., Tas, S.W. (2015), The contribution of NF-kappaB signalling to immune regulation and tolerance. *Eur J Clin Invest* 45: 529-539, 10.1111/eci.12430

Wang, Y., Sun, B., Volk, H.D., et al. (2011), Comparative study of the influence of proteasome inhibitor MG132 and ganciclovir on the cytomegalovirus-specific CD8(+) T-cell immune response. *Viral Immunol* 24: 455-461, 10.1089/vim.2011.0038

Weih, F., Carrasco, D., Durham, S.K., et al. (1995), Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. *Cell* 80: 331-340,

Yu, A., Malek, T.R. (2001), The proteasome regulates receptor-mediated endocytosis of interleukin-2. *J Biol Chem* 276: 381-385, 10.1074/jbc.M007991200


## Relationship: 2004: Suppression of T cell activation leads to Impairment, Impairment of TDAR

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Impaired IL-1R1 signaling leading to Impaired T-Cell Dependent Antibody Response | adjacent | High | High |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Homo sapiens | Homo sapiens | High | NCBI |
| Mus musculus | Mus musculus | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

### Key Event Relationship Description

Normal T cell and B cell function is indispensable for host defense mechanism. Various Interleukins (ILs) such as IL-2 and IL-4 are produced and secreted by activated helper T cells and play important roles in the development of T-cell dependent antibody response (TDAR). IL-4 affects maturation and class switching of B cells as well as proliferation, IL-2 promotes differentiation of B cells through IL-2 receptors and stimulates the activated T cell into T cell called Th2 cell. Therefore, suppressed production of IL-2 and IL-4 impairs T cell dependent antibody production (Alberts et al. 2008).

T cells, B cells, and antigen-presenting cells such as dendritic cells are involved in inducing and developing of TDAR. Thus, changes in any of these immune cell populations can influence TDAR. Activated T cell-derived cytokines play important roles in the development of TDAR. Among them, IL-2 promotes proliferation of B cells, and IL-4 affects maturation and class switching of B cells as well as proliferation, both of which induces/enhances T cell dependent antibody production.

Thus, suppressing the production of IL-2, IL-4, and other cytokines in T cells reduces stimulation of B cells including proliferation, activation, and class switching, and leading to impairment of TDAR. Therefore, suppressing the production of these B-cell-related cytokines appears to be the main factor in impairment of TDAR by inhibitors of T-cell–dependent-antibody production.

### Evidence Supporting this KER

In cynomolgus monkeys, the effects of CsA on production of IL-2 and IL-4, and antigen-specific IgM and IgG in TDAR were demonstrated (Gaida K. 2015).

Suppressed IgE and antigen specific IgG1 productions by the blocking of IL-4 receptor were reported in mice using dupilumab (antiIL-4/13R antibody) (Sanofi K.K. 2018).

Suppressed antigen specific IgE production by the inhibition of IL-4 production was reported in mice using suplatast tosilate (Taiho Pharmaceutical 2013). Suppressed antigen specific IgE and IL-4 productions by the inhibition of IL-4 production were reported in human cell culture using suplatast tosilate(Taiho Pharmaceutical 2013).

The effects of FK506 on serum concentration of anti-KLH antibodies IgM and IgG have been demonstrated in rats treated with FK506 for over four weeks and immunized with KLH (Ulrich et al. 2004).

The effects of FK506 and CsA on antigen-specific plaque-forming splenocytes have been demonstrated in mice treated with FK506 or CsA for 4 days and immunized with SRBC (Kino et al. 1987b).

The effects of FK506 and CsA on the levels of IgM and IgG in the culture supernatant have been demonstrated in human cells (Heidt et al, 2009, Sakuma et al, 2001). The effects of FK506 and CsA on production of IL-2 and IL-4 have been demonstrated using mice and human cells (Kino et al. 1987a, Dumont et al. 1998).

These facts suggest that there are no species differences between humans, monkeys and rodents in inhibitions of IL-2 and IL-4 production and TDAR induction.

**Biological Plausibility**

Cyclosporin A (CsA) is known to be one of the calcineurin inhibitiors. CsA-treatment is reported to suppress the productions of IL-2

and IL-4 and result in the reduction of the productions of antigen-specific IgM and IgG in cynomolgus monkeys (Gaida K. 2015).

It is established that IL-2 stimulates B cells to proliferate through the surface IL-2 receptors and that IL-4 stimulates B cells to proliferate, to induce class switch, and to differentiate into plasma and memory cells.

Dupilumab is known as anti-IL-4/13 receptor (IL-4/13R) antibody. Dupilumab (Dupixent) reduces productions of immunoglobulin (Ig)E and antigen specific IgG1 in mice (Sanofi K.K. 2018). It suggests that the blocking of IL-4 signaling by anti-IL-4/13R antibody results in the decrease in T cell dependent antibody production.

Th2 cell produces cytokines including IL-4. Suplatast tosilate (IPD) is known as an inhibitor of the production of IL-4 and IL-5 from Th2 cells and reduces the production of antigen specific IgE in human cell culture and mice (Taiho Pharmaceutical 2013). These findings suggests that the reduction of IL-4 production by the inhibitor of Th2 cell cytokines results in reduced production of IgE and/or IgG1 through inhibitions of maturation, proliferation and class switching of B cells.

IL-2 binds to IL-2 receptor (IL-2R) and acts on T cell. CD25 is one of IL-2R. Basiliximab (Simulect) is known as anti-CD25 antibody. Basiliximab binds to IL-2R and blocks IL-2 signaling. Clinical transplantation study of basiliximab reveals decreases in rejections. On the other hand, basiliximab inhibits the activation of antigen specific T cells (Novartis Pharma 2016). They suggest that the blocking of IL-2 signaling by anti-IL-2R antibody results in decreased rejection through the inhibition of the activation of antigen specific T cell with reduced antibody production.

FK506 and CsA suppress mRNA expression levels of cytokines in T cells including IL-2 and IL-4 that stimulate proliferation of B cells as well as B cell activation and class switching (Heidt et al, 2010).

Several in vivo studies in rodents showed decreased TDAR by the treatment of FK506 (Kino et al. 1987b, Ulrich et al. 2004). In in vitro tests examining antibody production in blood samples obtained from blood-bank donors, peripheral blood mononuclear cells (PBMC) treated with FK506 and CsA suppressed the production of IgM and IgG antibodies to T-cell dependent antigens (Heidt et al, 2009).

T cells, B cells, and antigen-presenting cells such as dendritic cells are involved in inducing and developing of TDAR. Thus, changes in any of these immune cell populations can influence TDAR. However, as for the suppression of humoral immunity induced by the inhibition of calcineurin (CN) phosphatase activity, calcineurin inhibitors (CNIs) do not affect B cells directly but rather indirectly through T cells. That is, FK506 and CsA are capable of inhibiting immunoglobulin production when B cells are cultured with non-pre-activated T cells, but FK506 and CsA fail to inhibit immunoglobulin levels when pre-activated T cells are used to stimulate B cells. Hence, the inhibition of B cell response by FK506 and CsA appears due solely to inhibition of T helper cells (Heidt et al, 2010).

Therefore, it is concluded that decreased amounts of IL-2 and IL-4 secreted from helper T cells is the main factor for suppression of TDAR induced by CN phosphatase inhibition.

**Empirical Evidence**

Empirical support of the suppression, IL-2 and IL-4 production leads to impairment, T-cell dependent antibody response is strong.

Rationale

- Cynomolgus monkeys treated wth CsA at 50 mg/kg BID for 24 days suppression of IL-2, IL-4 and sheep red blood cell (SRBC)-specific IgM and IgG (Gaida K. 2015).
- In the allergen-induced pneumonia model in mice, dupilumab (anti-IL-4/13R antibody) reduced productions of IgE and antigen specific IgG1 at 25 mg/kg of twice weekly subcutaneous administration for 4weeks (Sanofi K.K. 2018).
- In mice immunized with dinitrophenyl antigen by i.p. injection, suplatast tosilate (an inhibitor of the production of cytokines on Th2 cell) reduced productions of antigen specific IgE at 10, 20, 50 and 100 mg/kg of oral administration for 5 days (Taiho
- Pharmaceutical 2013). In human cell culture immunized with Japanese cedar antigen, suplatast tosilate reduced productions of antigen specific IgE at the concentration of 10 µg/mL for 10 days (Taiho Pharmaceutical 2013).
- In the clinical study of renal transplantation, basiliximab decreased incidence of acute rejection at 20 mg/kg (Novartis Pharma 2016). In human T cell culture immunized with PPD, basiliximab reduced activation of antigen specific T cell at the

concentration of 300 ng/mL (Novartis Pharma 2016).
- In CD3/phorbol 12-myristate-13-acetate-activated human T cells, FK506 suppressed production of IL-2, IL-4 and Interferon (IFN)-γ at the concentrations of 1.2 to 12.5 nM as well as inhibited expression of IL-2, IL-4 and IFN-γ mRNA at the
- concentrations of 10 nM. (Dumont et al. 1998).
- FK506 or CsA suppressed production of IL-2 in mouse mixed lymphocyte reaction (MLR) at 0.1 to 10 nM of FK506 and 10 to 100 nM of CsA as well as in human MLR at 0.1 to 10 nM of FK506 and 10 to 100 nM of CsA (Kino et al. 1987a).
- After 9-day culture of B cells and non-pre-activated T cell stimulationwith FK506 or CsA, the levels of IgM and IgG in the culture supernatant were reduced at 0.3 and 1.0 ng/mL (0.37 and 1.24 nM) of FK506 or 50 and 100 ng/mL (41 and 83nM) of CsA (Heidt et al, 2009).
- After 4-day culture of SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) and anti-CD3/CD28 stimulated PBMC culture supernatant with FK506 or CsA, the level of IgM in the culture supernatant was reduced at the concentrations of 0.01 to 100 ng/mL (0.01 to 124 nM) of FK506 or 0.1 to 1000 ng/mL (0.08 to 832 nM) of CsA (Sakuma et al, 2001).
- Rats were treated with FK506 for over four weeks and immunized with keyhole limpet hemocyanine (KLH), after which serum concentration of anti-KLH IgM and IgG reduced at the dose levels of 3 mg/kg/day (Ulrich et al. 2004).
- Mice were treated with FK506 or CsA for 4 days, and immunized with sheep red blood cells (SRBC), after which antigenspecific plaque-forming splenocytes reduced at the dose levels of 3.2, 10, 32 and 100 mg/kg of FK506 or 32 and 100 mg/kg of CsA (Kino et al. 1987b).
- 1,2:5,6-dibenzanthracene single administration suppressed production of IL-2 and total IgG antibody in mice at the dose levels of 3 and 30 mg/kg(Donna, C. et al. 2010).
- In male CD-1 mice, chronic psychosocial stress (types of social outcome occurred: residents becoming subordinates) for 21 days reduced IL-2 release in response to KLH and decrease in anti-KLH IgG (Alessandro, B. et al. 2003).

**Uncertainties and Inconsistencies**

IL-2 affects multiple populations of immune cells expressing IL-2 receptors, while IL-4 mainly acts on B cells. Therefore, reduced production of both IL-2 and IL-4 might certainly induce suppression of TDAR; however, there remains some possibility of additional suppression of other immune functions.

### Quantitative Understanding of the Linkage

Luster et al (1993) demonstrated that Concanavalin A response of splenocytes showed the linear dose-response relationship with the host resistnace to Listeria monocytogenes or Streptococcus pneumoniae.

**Response-response relationship**

Cynomolgus monkeys treated wth CsA at 50 mg/kg BID showed suppression of IL-2 and IL-4 production and inhibition of SRBC-specific IgM and IgG in TDAR (Gaida K. 2015).

In the blocking of IL-4 receptor in mice by dupilumab (anti-IL-4/13R antibody) at 25 mg/kg of twice weekly subcutaneous administration for 4weeks, IgE production was suppressed to about 1/100 and antigen specific IgG1 production was suppressed to about 1/200 (Sanofi K.K. 2018).

In the inhibition of IL-4 production in mice by suplatast tosilate at 10, 20, 50 and 100 mg/kg of oral administration for 5 days, antigen specific IgE production was suppressed from about 1/10 to 1/100 (Taiho Pharmaceutical 2013). In human T cell culture by suplatast tosilate at the concentration of 10 µg/mL, antigen specific IgE production after 10 days was suppressed from 56 to 72% and IL-4 production after 3 days was suppressed from 58 to 76% (Taiho Pharmaceutical 2013).

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. That is, murine small resting B cells, cultured with irradiated hapten-specific TH1 clone, were induced to enter cell cycle at 2 days and to secret antibody at 5 days. An anti-IL-2 and anti-IL-2R antibodies completely inhibited this T-cell dependent antibody production (Owens T, 1991).

In the human T-B cell co-culture stimulated with anti-CD3 monoclonal antibody, CNIs of FK506 and CsA lowered the m-RNA levels of T-cell cytokines at 8h post-stimulation including IL-2 and IL-4 at 1.0ng/mL (1.24nM) FK506 or 100ng/mL (90.7nM) CsA and inhibited IgM and IgG productions after 9 days at 0.3 and 1.0ng/mL FK506 and 50 and 100ng/mL CsA (Heidt S. 2010).

**Time-scale**

In CsA-treatment for 24 days at 50 mg/kg BID, cynomolgus monkeys showed suppression of IL-2 and IL-4 production and inhibition of SRBC-specific IgM and IgG in TDAR (Gaida K. 2015).

In human T cell culture, suplatast tosilate inhibits IL-4 production after 3 days and antigen specific IgE production after 10 days (Taiho Pharmaceutical 2013).

In the human T-B cell co-culture, CNIs of FK506 and CsA lowered the m-RNA levels of IL-2 and IL-4 at 8h post-stimulation and inhibited IgM and IgG productions after 9 days (Heidt S. 2010).

**Known Feedforward/Feedback loops influencing this KER**

At present, no evidence is found.

## References

Alberts, B., Johnson, A., Lewis, L., Raff, M., Roberts, K. and Walter, P. (2008). Molecular Biology of the Cell. 5th ed., Garland Science, New York. 1539-1601

Sanofi K.K. (2018) Drug interview form Dupixent subcutaneous injection 300 mg syringe. 2nd edition.

Taiho Pharmaceutical Co.,Ltd. (2013) Drug interview form IPD capsule 50 and 100. Revised 5th edition.

Novartis Pharma K.K. (2016). Drug interview form Simulect i.v. injection 20 mg. 10th edition.

Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.

Heidt, S., Roelen, D. L., Eijsink, C., Eikmans, M., van Kooten, C., Claas, F. H. and Mulder, A. (2010). Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. Clinical and experimental immunology. 159(2): 199- 207. Gaida K., Salimi-Moosavi H., Subramanian R., Almon V., Knize A., Zhang M., Lin F.F., Nguyen H.Q., Zhou L., Sullivan J.K., Wong M., McBride H.J. (2015). Inhibition of CRAC with a human anti-ORAI1 monoclonal antibody inhibits T-cell-derived cytokine production but fails to inhibit a T-cell-dependent antibody response in the cynomolgus monkey. J Immunotoxicol AOP154 38/39 12:164-173.

Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., Goto, T., Okuhara, M., Kohsaka, M. and Aoki, H. (1987a). FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. Journal of antibiotics. 40(9): 1256-1265.

Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Imanaka, H. (1987b). FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. Journal of antibiotics. 40(9): 1249-1255.

Owens T.(1991). Requirement for noncognate interaction with T cells for the activation of B cell immunoglobulin secretion by IL-2. Cell Immunol 133:352-366.

Sakuma, S., Kato, Y., Nishigaki, F., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2001b). Effects of FK506 and other immunosuppressive anti-rheumatic agents on T cell activation mediated IL-6 and IgM production in vitro. International Immunopharmacology 1(4): 749-57.

Ulrich, P., Paul, G., Perentes, E., Mahl, A., and Roman D. (2004). Validation of immune function testing during a 4-week oral toxicity study with FK506. Toxicology Letters 149(1-3): 123-31.

Alessandro B, Paola S, Alberto E. Paneraic, Tiziana P,Paola Palanzaa and Stefano P(2003). Chronic psychosocial stress-induced down-regulation of immunity depends upon individual factors Journal of Neuroimmunology 141: 58–64

Donna C. S, Matthew J. S and Kimber L. W Jr. (2010) Systemic immunosuppression following a single pharyngeal aspiration of 1,2:5,6-dibenzanthracene in female B6C3F1 mice, Journal of Immunotoxicology, 7:3, 219-231