厚生労働行政推進調査事業費補助金（化学物質リスク研究事業）

# OECD プログラムにおいて TG と DA を開発するための AOP に関する研究

平成30年度 分担研究報告書

## 免疫毒性試験の TG および免疫毒性 AOP 開発

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**研究要旨**

現在、厚労科研（化学物質の動物個体レベルの免疫毒性データ集積とそれに基づくMulti-ImmunoTox assay（MITA）による予測性試験法の確立と国際標準化（H30-化学-一般-001））にて、MITAのOECDテストガイドライン化に向けてのvalidation試験を実施中である。MITAのテストガイドライン化に際しては、その理論的根拠となるAOPの作成が不可欠である。ガイドライン化を予定しているMITAの試験項目は、化学物質によるT細胞のIL-2転写抑制評価系と単球のIL-1転写抑制評価系である。前者に関しては、既に本厚労科研において足利らがInhibition of calcineurin activity leading to impaired T-cell dependent antibody response (AOP:154)を作成中であり、後者に関して、今年度AOPを（Aop:277）を作成した。今回、2019年3月25日国立医薬品食品衛生研究所にて、OECDのAOPプログラムの現在座長でありEuropean Commission, Joint Research Center, ItalyのMaurice Whelan先生から直接作成中のAOPに関して意見を伺うことができ、それを考慮してAOPを修正した。次年度中に、EAGMSTによるreviewを予定している。

## A. 研究目的

環境中に存在する何万という化学物質のなかには、免疫系を標的として健康被害を及ぼすものが多数存在する。したがって、免疫毒性は、消費者、生産者はもとより公衆衛生行政にとっても重要な課題となっている。現在、免疫毒性評価は動物実験を用いて行われているが、数万ともいわれる化学物質を網羅的に評価、管理するには、動物を用いない評価手法の開発が喫緊の課題である。その際、最終的にはQSARやカテゴリーアプローチ等の予測的評価法の開発が必須であるが、そのためにも免疫毒性AOPの作成とそれに基づいた high throughput screening(HTP)法の確立が不可欠である。一方、我々はこれまでに多項目免疫毒性評価系 (MITA)を開発し、その data set の作成、有用性の検討、国際標準化へむけてのvalidation 等を行ってきた。その中で、60 種類の化学物質を同じく我々が開発し OECD テストガイドラインに承認されている皮膚感作性試験 IL-8 Luc assay と MITA を組み合わせた modified mMITA により評価し、それらを複数のパラメータに関する効果発現最低濃度 (Lowest observed effect level ; LOWEL)を基にクラスター分類することにより、免疫毒性物質が 6 種類のクラスターに分類できることを明らかにした。そこで、

本課題では mMITA を多項目免疫毒性評価系として OECD テストガイドライン化することを目標に、その理論的背景となる adverse outcome pathway を作成する。

**B. 研究方法**

B.1. mMITA を評価系として用いる AOP の構築 (H30)

我々がこれまでに開発した MITA は、T 細胞の IL-2、IFN-γ のプロモーター活性、単球の IL-1β、IL-8 プロモーター活性に与える化学物質の影響をルシフェラーゼ活性により high throughput に評価することができる（Kimura et al. Toxicol in Vitro, 2015）。さらに、これに IL-8 Luc assay を加えた mMITA では化学物質の皮膚感作性も評価できる。今年度、人体への影響が明らかな免疫抑制剤を含む 60 種類の化学物質を評価した data set を作成した。そこで、MITA の 4 種類のパラメータの内の２つと IL-8 Luc assay を用いて化学物質の免疫毒性による hierarchical clustering を施行した。その結果、化学物質が最大 6 つのクラスターに分けられることが明らかになった (Kimura et al. Arch Toxicol,2018)。これまでに化学物質の免疫毒性を clustering の手法で評価しようという試みの報告はない。そこで、現在、厚労科研（化学物質の動物個体レベルの免疫毒性データ集積とそれに基づく Multi-ImmunoTox assay（MITA）による予測性試験法の確立と国際標準化（H30-化学-一般-001））にて、MITA の OECD テストガイドライン化に向けての validation 試験を実施中である。MITA のテストガイドライン化に際しては、その理論的根拠となる AOP の作成が不可欠である。ガイドライン化を予定している MITA の試験項目は、化学物質による T 細胞の IL-2 転写抑制評価系と単球の IL-1 転写抑制評価系である。本研究では特に後者に関して AOP を作成する。

B.2. AOP の国際的認証 (H32)

完成した AOP は AOP WIKI（https://aopwiki.org/wiki/index.php/Main_Page)にアップロードし、最終的には the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST)による承認を目指す。まず、各 AOP に関して、AOP Title, Authors, Abstract, Background, Summary of the AOP, Graphical Representation, Overall Assessment of the AOP, References の形式に沿って記載し AOP WIKI にアップロードする。

（倫理面への配慮）
特に必要とされない。

**C. 研究結果**

C.1. Inhibition of IL-1 signalingのAOP作成

本年度は、Inhibition of IL-1 signalingに関するAOPを作成した (Aop: 277)。AOP WIKIの記載要項に沿って作成した原稿をAppendix 1 AOP for inhibition of IL-1 signalingに示す。また、この内容に関して、OECDのAOPプログラムの現在座長であり European Commission, Joint Research Center, Italy の Maurice Whelan先生のご意見と伺うことができた {2019年3月25日国立医薬品食品衛生研究所}。Whelan先生は、OECDのAOPプログラムの現在座長を務めていられる方で、AOPの作成上の注意点などを直接伺いAOP作成の参考にした。

C.2. Inhibition of IL-1 signaling AOP の AOP WIKI への登録

作成した AOP を AOP WIKI に登録している (AOP 277).

**D. 考察**

現在、他の厚労科研、化学物質の動物個体レベルの免疫毒性データ集積とそれに基づくMulti-ImmunoTox assay（MITA）による予測性試験法の確立と国際標準化（H30-化学-一般-001）にて, MITAのOECDテストガイドライン化に向けてのvalidation試験を実施中である。申請に際して必要となるvalidation reportの作成において、MITA評価項目に関連するAOPの存在は不可欠である。ガイドライン化を予定しているMITAの試験項目は、化学物質によるT細胞のIL-2転写抑制, 単球のIL-1転写抑制の評価系である。前者に関しては，既に本厚労科研において足利らがInhibition of calcineurin activity leading to impaired T-cell dependent antibody response (AOP: 154)を作成中であり，後者に関しては我々が作成中のAop:277)が対応する。

今回，幸いに OECD の AOP 担当座長である Whelan 先生から作成中の AOP に関して意見を伺うことができた。意見は概ね本 AOP に肯定的で、How many Key Events (KEs) are required in the AOP and what is required in Key Event Relationship when the signaling pathway is confirmed?という質問に関して、KEs are prepared for each step, such as macromolecular, cell/Tissue, Organ/Organ system, individual.

Even though the pathway is confirmed, the information regarding empirical evidence, uncertainties and inconsistencies, response-response relationship, time scale should be described.

などの貴重な意見を頂いた。

引用文献

Kimura, Y., Fujimura, C., Ito, Y., Takahashi, T., Terui, H., Aiba, S., 2018. Profiling the immunotoxicity of chemicals based on in vitro evaluation by a combination of the Multi-ImmunoTox assay and the IL-8 Luc assay. Arch Toxicol. 92, 2043-2054.

Kimura, Y., Fujimura, C., Ito, Y., Takahashi, T., Nakajima, Y., Ohmiya, Y., Aiba, S. Optimization of the IL-8 Luc assay as an in vitro test for skin sensitization. Toxicol In Vitro 2015. 29, 1816-1830

**E. 結論**

Inhibition of IL-1 signalingのAOPを作成し、AOP WIKIに登録中である。また、OECDのAOP担当座長であるWhelan先生から貴重な意見を伺え、それを参考にしてさらなる改善をはかっている。

**F. 研究発表**

F.1. 論文発表

1. Kimura, Y., Fujimura, C., Ito, Y., Takahashi, T., Terui, H., Aiba, S., 2018a. Profiling the immunotoxicity of chemicals based on in vitro evaluation by a combination of the Multi-ImmunoTox assay and the IL-8 Luc assay. Arch Toxicol 92, 2043-2054.
2. Kimura, Y., Watanabe, M., Suzuki, N., Iwaki, T., Yamakage, K., Saito, K., Nakajima, Y., Fujimura, C., Ohmiya, Y., Omori, T., Kojima, H., Aiba, S., 2018b. The performance of an in vitro skin sensitisation test, IL-8 Luc assay (OECD442E), and the integrated approach with direct peptide reactive assay (DPRA). J Toxicol Sci 43, 741-749.

F.2. 学会発表

1. 木 村 裕 他 ： Multi-ImmunoTox Assay (MITA) ：バリデーション研究の結果 日本動物実験代替法学会 第31回大会（熊本） 2018年11月

## G．知的財産権の出願・登録状況

G.1. 特許取得

なし

## H. 添付資料

AOP for inhibition of IL-1 signaling

# AOP for inhibition of IL-1 signaling

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**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. The activation of NF-κB plays a principle 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.

In this AOP, we considered 2 MIEs, such as blocking IL-1 R and decreased IL-1 production. Either MIE leads to reduced IL-1 signaling. The biological plausibility of the signaling cascade from the activation of IL-1R to the activation of NF-κB is already confirmed. In addition, the biological plausibility that suppressed NF-κB activation leads to impaired T cell activation, resulting in impaired antibody production and that impaired T cell function and antibody production lead to increased susceptibility to infection is supported by quite a few published works.

IL-1 also mediates several autoinflammatory syndromes. Therefore, several inhibitors against IL-1 signaling such as IL-1Ra (generic anakinra) , canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) have been developed. After these inhibitors became available to treat these disorders, it became clear that these inhibitors increased the frequency of serious bacterial infection. Similarly, the experiments using knockout mice revealed that the lack of IL-1 signaling led to bacterial, tuberculosis or viral infection. Beside the blocking of IL-1 binding to its receptor, several drugs also suppress the production of IL-1. Dexamethasone is one of the representatives that significantly suppress IL-1β production from monocytes. Although the effects of dexamethasone are pleiotropic, it is well known to increase the susceptibility to bacterial, fungal, or viral infection. Minocycline or two caspase-1 inhibitors, Pralnacasan (VX-740) and Belnacasan (VX-765, also HMR3480 that are orally absorbed compounds and synthetized as prodrugs which are then converted into the

active principle, VRT-018858 and VRT-043198, respectively also suppress IL-1 signaling by the inhibition of caspase-1 activation, which is an essential enzyme for maturation of pro- IL-1β and the secretion of mature IL-1β. Recently, it has been reported that cinnamicaldehyde suppresses serum IL-1β level in endotoxin poisoning mice. These data suggest that chemicals as well as drugs can suppress IL-1 signaling through their inhibitory effects on IL-1β. Taken together, developing the AOP for inhibition of IL-1 signaling is mandatory.

**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 against infection, 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 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).

IL-1 also mediates autoinflammatory, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever. Consequently, IL-1 family cytokines have sophisticated regulatory mechanisms to control their activities including proteolytic processing for their activation and the deployment of soluble receptors and receptor antagonists to limit their activities. Therefore, several inhibitors against IL-1 signaling have been developed. IL-1 receptor antagonist（IL-1Ra）was purified in 1990, and the cDNA was 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 was 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. 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 also reported the increased susceptibility to bacterial or tuberculosis infection (Genovese et al., 2004; Kullenberg et al., 2016; Lequerre et al., 2008; Migkos et al., 2015). As IL-1 signaling antagonists, two drugs went up to the market, canakinumab (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; Imagawa et al., 2013; Lachmann et al., 2009; 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).

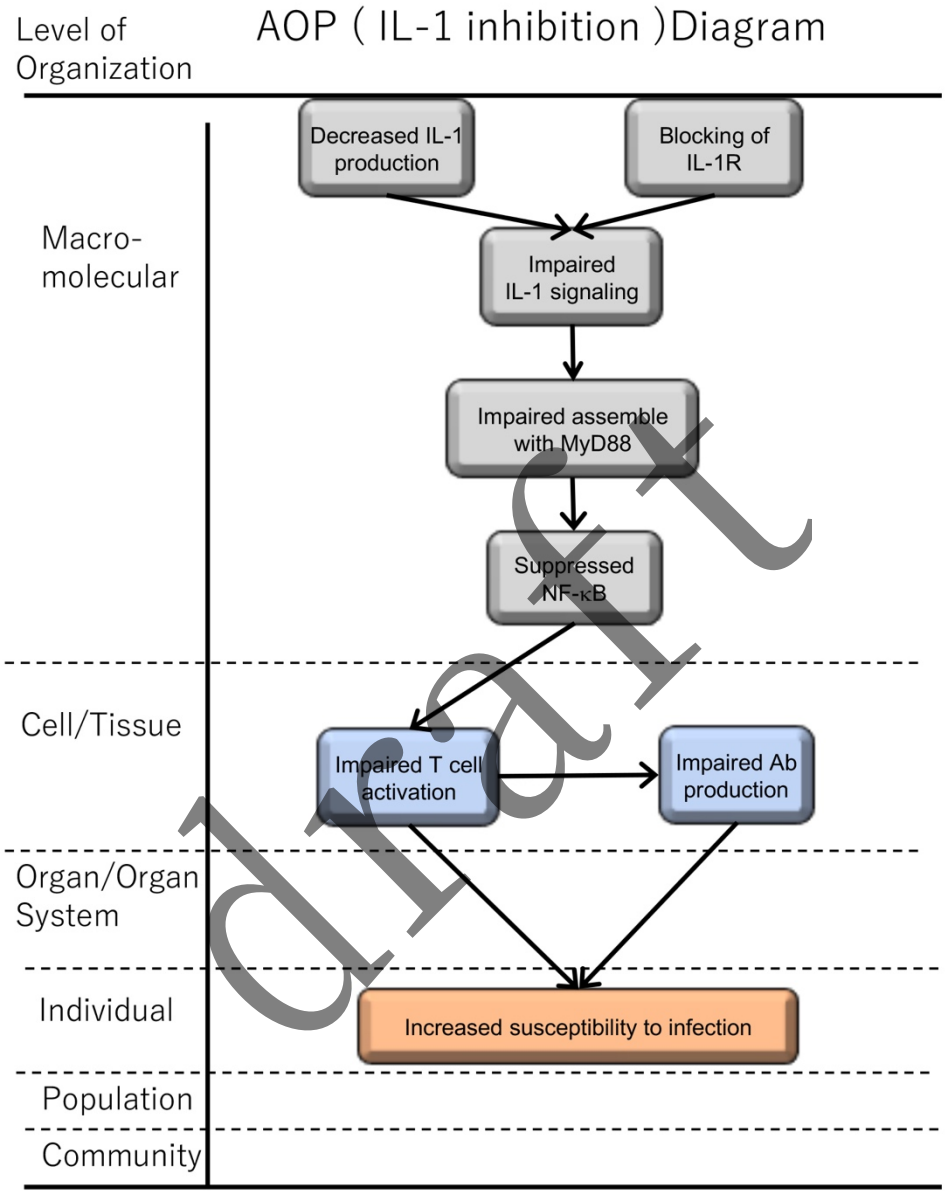
Beside the blocking of IL-1 binding to its receptor, several drugs also suppress the production of IL-1. Dexamethasone is one of the representatives that significantly suppress IL-1β production from monocytes (Finch-Arietta and Cochran, 1991). Minocycline, and pralnacasan (VX-740) and belnacasan (VX-765) that are orally absorbed compounds and synthetized as prodrugs which are then converted into the active principle, VRT-018858 and VRT-043198, respectively (Fenini et al., 2017) also suppress IL-1 signaling by the inhibition of caspase-1 activation, which 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). These data suggest that chemicals as well as drugs can suppress IL-1 signaling through their inhibitory effects on IL-1β.

In this AOP, we considered 2 MIEs, such as blocking IL-1 R and decreased IL-1 production. Either MIE leads to reduced IL-1 signaling. The biological plausibility of the signaling cascade from the activation of IL-1R to the activation of NF-κB is already accepted. In addition, the biological plausibility that suppressed NF-κB activation leads to impaired T cell activation, resulting in impaired antibody production and impaired T cell and antibody production lead to increased susceptibility to infection is confirmed.

Moreover, Patients with defects in MyD88 gene have an increased susceptibility to pyogenic bacterial infections (Picard et al., 2010; von Bernuth et al., 2008)(von Bernuth et al. 2008, Picard et al. 2010). The fact that MyD88 knockout mice showed fatal mycobacterium tuberculosis infection supports the significance of MyD88. (Fremond et al., 2004; Scanga et al., 2004).

These data suggest that IL-1 signaling via MyD88 is indispensable for the defense against microorganisms, and assessment of IL-1 signaling is a good tool for screening the chemical that influence to the host defense.

**Summary of the AOP**






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

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| | MIE | 1570 | Blocking of IL-1R | Blocking of IL-1R |

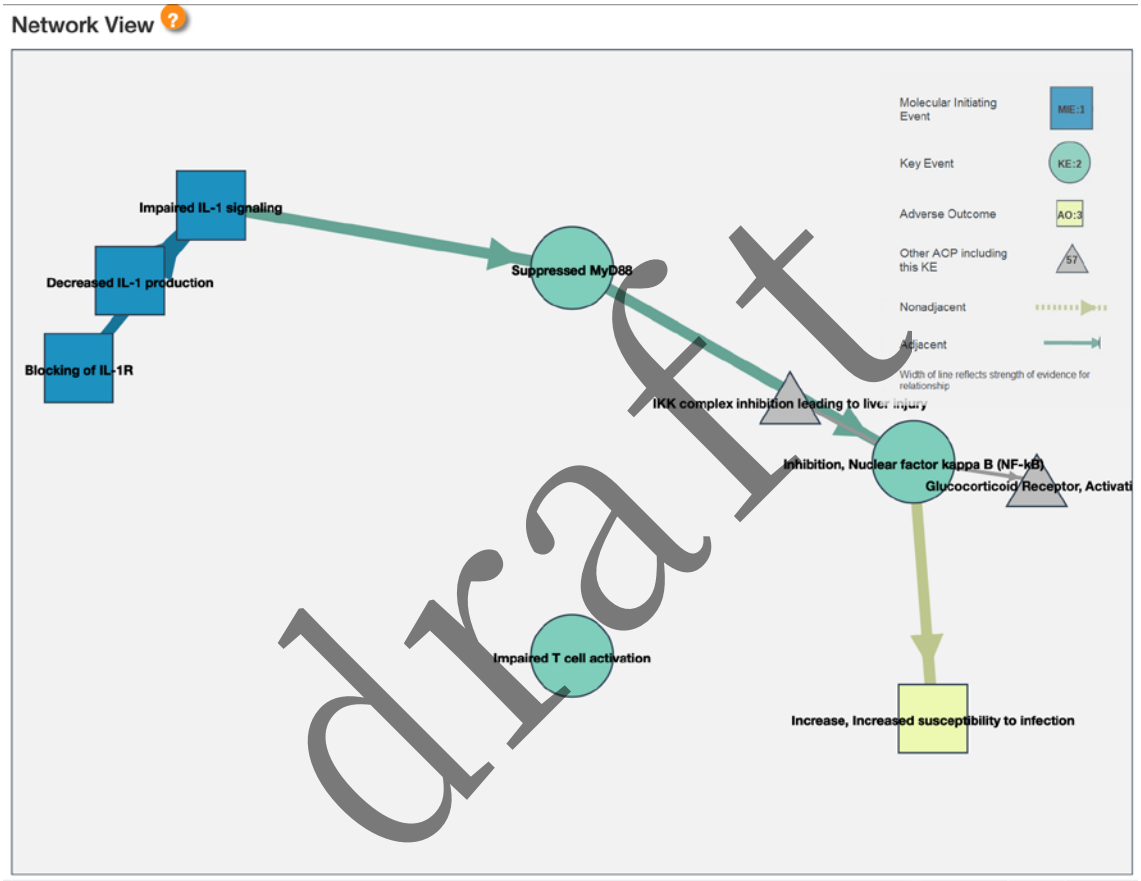
| | | | | |
|---|---|---|---|---|
| | MIE | 1571 | Decreased IL-1 production | Decreased IL-1 production |
| | MIE | 1572 | Impaired IL-1 signaling | Impaired IL-1 signaling |
| | | | | |
| | KE | 202 | Inhibition, Nuclear factor kappa B (NF-kB) | Inhibition, Nuclear factor kappa B (NF-kB) |
| | | | | |
| | AO | 986 | Increase, Increased susceptibility to infection | Increase, Increased susceptibility to infection |
| | | | | |

**Relationships Between Two Key Events (Including MIEs and AOs)**

| Title | Adjacency | Evidence | Quantitative Understanding |
|---|---|---|---|
| Blocking of IL-1R leads to Impaired IL-1 signaling | adjacent | High | High |
| Decreased IL-1 production leads to Impaired IL-1 signaling | adjacent | High | High |
| Impaired IL-1 signaling leads to Suppressed MyD88 | adjacent | High | High |
| Suppressed MyD88 leads to Inhibition, Nuclear factor kappa B (NF-kB) | adjacent | High | High |
| nhibition, Nuclear factor kappa B (NF-kB) leads to | adjacent | High | High |

| | | | |
|---|---|---|---|
| Increase, Increased susceptibility to infection | | | |
| | | | |

Network View




Stressors

Dexamethosone, minocycline, two caspase-1 inhibitors, Pralnacasan (VX-740) and Belnacasan (VX-765, also HMR3480, cinnamic aldehyde, IL-1 receptor antagonist（IL-1Ra）(Anakinra), anti-IL-1b antibod (Canakinumab), soluble IL-1R (Rilonacept).

**Life Stage Applicability**
**Taxonomic Applicability**
**Sex Applicability**
**Overall Assessment of the AOP**
**Domain of Applicability**

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).

These data suggest that the proposed AOP regarding inhibition of IL-1 signaling is not dependent on life stage, sex, age or species.



**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 (Guler et al., 2011; Horino et al., 2009; Juffermans et al., 2000; Tian et al., 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; Migkos et al., 2015). 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 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. Although MyD88 is also known to be involved in TLR signaling pathway, several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated. These data suggest to essentiality of IL-1-MyD88 signaling pathway in host defense against infection.

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

**Evidence Assessment**

The recent review of IL-1 pathway by Weber et al. has clearly described the intracellular signaling event from the binding of IL-1a or IL-1b to IL-1R to the activation of NF-kB through the assemble of MyD88 to the trimelic 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 (Weber et al., 2010a, b).

KER1:Blocking of IL-1R leads to Impaired IL-1 signaling.

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 et al. 1990), IL-6 (Goh et al. 2014), and T cell proliferation (Seckinger et al. 1990).

KER2: Decreased IL-1 production leads to Impaired IL-1 signaling.

**Quantitative Understanding**

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

**MIE: Title: Inhibition of IL-1 binding to IL-1R**

**Short name: Inhibition of IL-1 binding to IL-1R**

**Biological organization: Molecular**

**Cell term: Macrophage**

**Organ term: immune system**

**Stressors**

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

**Key event description**

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-R like rilonacept (Kapur and Bonk, 2009). The binding of IL-1β to IL-1R1 can be inhibited by anti-IL-1β antibody (anti-IL-1β antibody) (Church and McDermott, 2009).

**How it is measured or detected.**

1. Competitive inhibition binding experiments using $^{125}$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 (McIntyre et al., 1991; Shuck et al., 1991).
2. Measure the ability of the reagent to neutralize the bioactivity of human IL-1β on primary human fibroblasts in vitro (Alten et al., 2008)

**Applicability domain**

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).

These data suggest that the proposed AOP regarding inhibition of IL-1 signaling is not dependent on life stage, sex, age or species.

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

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

**MIE: Title: Decreased IL-1 production**

**Short name: Decreased IL-1 production**

**Biological organization: Molecular**

**Cell term: Macrophage**

**Organ term: immune system**

**Stressors:** Chemical:20384 Dexamethasone, minocycline, pralnacasan (VX-740), belnacasan and cinnamic aldehyde

**Key event description**

Decreased IL-1 production by macrophages can be induced by suppressed IL-1 b mRNA induction or suppressed maturation of pro-IL-1b which leads to decreased IL-1b secretion. Dexamethasone is one of the representative drugs that significantly suppress IL-1β production from monocytes (Finch-Arietta and Cochran, 1991). Minocycline, and pralnacasan (VX-740) and belnacasan (VX-765) that are orally absorbed compounds and synthetized as prodrugs which are then converted into the active principle, VRT-018858 and VRT-043198, respectively (Fenini et al., 2017) also suppress IL-1 signaling by the inhibition of caspase-1 activation, which 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). These data suggest that chemicals as well as drugs can suppress IL-1 signaling through their inhibitory effects on IL-1β production.

**How it is measured or detected.**

Inhibition of IL-1 mRNA expression is measured by quantitative real-time polymerase chain reaction.

The production of IL-1b is measured by ELISA (Karpenko et al., 2018).

**Applicability domain**

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).

These data suggest that the proposed AOP regarding inhibition of IL-1 signaling is not dependent on life stage, sex, age or species.

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

Dexamethasone inhibits IL-1b gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-kB/Rel and AP-1 activation (Jeon et al., 2000).
Dexamethasone suppress LPS-induced gene expression of IL-1 beta in rat lung. (in vivo) (Qiu et al., 1997)
DXM inhibited the release of IL-1b by human leukocyte stimulated with Streptococcus pneumoniae stimulation (van Furth et al., 1995).
Treatment of peripheral blood monocytes with 2 μg/ml lipopolysaccharide potently increased IL-1b release (p= 0.001) and dexamethasone ($10^{-7}$ M) significantly reduced both resting and stimulated IL-Ib release (p 0.009).) (Morand et al., 1993)
DEX a effectively blocks the glutamine antagonist acivicin-induced expression of IL-1b mRNA by HL-60 leukemia cells (Weinberg et al., 1992).

LPS treatment induced a significant upregulation of the mRNA and release of IL-1beta from retinal microglia. Minocycline inhibited its releases. Thus, minocycline might exert its antiinflammatory effect on microglia by inhibiting the expression and release of IL-1beta (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 VX-765 (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).
VX-765, an orally active IL-converting enzyme/caspase-1 inhibitor, blocked IL-1b secretion with equal potency in LPS-stimulated cells from FCAS and control subjects (Stack et al., 2005).

The study was intended to examine the protective effect of cinnamaldehyde (CM) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) mice model. The results of the investigation confirmed that, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were significantly decreased by CM (Huang and Wang, 2017).
The suppressing capacities of six cinnamaldehyde-related compounds were evaluated and compared by using the lipopolysaccharide (LPS)-primed and adenosine 5'-triphosphate (ATP)-activated macrophages. At concentrations of 25~100 μM, cinnamaldehyde and 2-methoxy cinnamaldehyde dose-dependently inhibited IL-1b 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).

**KE: Title: Impaired IL-1 signaling**

**Short name: Impaired IL-1 signaling**

**Biological organization: Molecular**

**Cell term: Macrophage**

**Organ term: immune system**

**Stressors:** Dexamethosone, minocycline, cinnamic aldehyde, IL-1 receptor antagonist（IL-1Ra）(Anakinra), anti-IL-1b antibod (Canakinumab), soluble IL-1R (Rilonacept).

**Key event description**

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 against infection, 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 (Dinarello, 2018; Weber et al., 2018). Therefore, decreased IL-1 production by macrophages, dendritic cells, epithelial cells, and endothelial cells or inhibition of IL-1 binding to IL-1R1 by anti-IL-1β antibody, IL-1RA, or soluble IL-1Ra1 inhibits the formation of the trimeric complex which results in impaired IL-1 signaling.

**How it is measured or detected.**

It is not possible to directly measure the activation of IL-1 signaling. Instead, the activation of IL-1 signaling can be indirectly measured by the activation of NF-kB or mRNA or protein expression of IL-1 responsive cytokines, such as IL-6 or IL-8, or cyclooxygenase 2.

NFκB p65 (Total/Phospho) ELISA：

ELISA for IL-6, IL-8, and Cox-2.

**Applicability domain**

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).

These data suggest that the proposed AOP regarding inhibition of IL-1 signaling is not dependent on life stage, sex, age or species.

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

IL-1 is known to mediates autoinflammatory syndrome, such as cryopyrin-associated periodic syndrome, neonatal-onset multisystem inflammatory disease and familial Mediterranean fever. The stressors of this MIE, such as anakinra, canakinumab, and rilonacept have been already used to treat these autoinflammatory syndrome associated with overactivation of IL-1 signaling reviewed by Gabay et al (Gabay et al., 2010).

Dexamethasone suppression of IL-1 beta-induced cyclooxygenase 2 expression is not mediated by lipocortin-1 in A549 cells (Newman et al., 1994).
Dexamethasone regulates IL-1 beta and TNF-alpha-induced interleukin-8 production in human bone marrow stromal and osteoblast-like cells (Dexamethasone ($10^{-7}$ M) significantly inhibited IL-lb plus TNF-a stimulated IL-8 production in HBMS, MG-63, and hOB cells (Chaudhary and Avioli, 1994).
Dexamethasone blocks the induction of IL-6 and IL-8 by IL-1-stimualted human lung fibroblasts (Monick et al., 1994).

VX-765, an orally active IL-converting enzyme/caspase-1 inhibitor, blocked IL-1b

secretion with equal potency in LPS-stimulated cells from FCAS and control subjects (Stack et al., 2005).

The study was intended to examine the protective effect of cinnamaldehyde (CM) on lipopolysaccharide (LPS)-induced acute lung injury (ALI) mice model. The results of the investigation confirmed that, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were significantly decreased by CM (Huang and Wang, 2017). Cinnamaldehyde reduced the neurological deficit scores, brain oedema and infarct volume. Cinnamaldehyde suppressed the activation of signal transduction molecules including toll-like receptor 4, tumour necrosis receptor-associated factor 6 and NF-κB, attenuated the increased levels of TNF-α, IL-1β, CCL2 and endothelial-leukocyte adhesion molecule-1 and ultimately reduced leukocyte infiltration into the ischaemic brain areas after cerebral ischaemia (Zhao et al., 2015).

**KE: Title: Inhibition, Nuclear factor kappa B (NF-κB)**

**Short name:** Inhibition, Nuclear factor kappa B (NF-κB)

**Biological organization: Molecular**

**Cell term: Macrophage**

**Organ term: immune system**

**Stressors:**

**Key event description**

The NF-κB pathway consists of a series of events 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-α. Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates IκBα. 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 IκBα 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. (Frederiksson 2012). (Gupta et al. 2010).(Huppelschoten 2017).(Liu et al. 2017).

**How it is measured or detected.**

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

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

NF-κB translocation: RelA-GFP reporter assay (Frederiksson 2012) (Huppelschoten 2017)

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

NF B p65 (Total/Phospho) ELISA:
ELISA for IL-6, IL-8, and Cox

**Applicability domain**

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). 17β-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**

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

References

Frederiksson, L., 2012. *TNFalpha-signaling in drug induced liver injury*. University of Leiden.

Gupta, S.C. et al., 2010. Inhibiting NF-??B activation by small molecules as a therapeutic strategy. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 1799(10–12), pp.775–787. Available at: http://dx.doi.org/10.1016/j.bbagrm.2010.05.004.

Huppelschoten, S., 2017. *Dynamics of TNFalpha signaling and drug-related liver toxicity*. Leiden University.

Liu, T. et al., 2017. NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, 2(March), p.17023. Available at: http://www.nature.com/articles/sigtrans201723.

Miller, S.C. et al., 2010. Identification of known drugs that act as inhibitors of NF-κB signaling and their mechanism of action. *Biochemical Pharmacology*, 79(9), pp.1272–1280. Available at: http://dx.doi.org/10.1016/j.bcp.2009.12.021.

Moujalled, D.M. et al., 2012. In mouse embryonic fibroblasts, neither caspase-8 nor cellular FLICE-inhibitory protein (FLIP) is necessary for TNF to activate NF-?B, but caspase-8 is required for TNF to cause cell death, and induction of FLIP by NF-?B is required to prevent it. *Cell Death and Differentiation*, 19(5), pp.808–815. Available at: http://dx.doi.org/10.1038/cdd.2011.151.

**KE: Title: Inhibition, Impaired T cell activation**

**Short name:** Inhibition, Impaired T cell activation

**Biological organization: Molecular**

**Cell term: T cell**

**Organ term: immune system**

**Stressors:**

**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 for solid organ transplantation. 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.

Recognition of MHC/peptide complexes by the TCR and the co-receptors results in T-cell activation (for a review, see [5]). Signalling via the TCR is further supported by co-stimulatory (e.g. CD28) and accessory (e.g. integrins) molecules. Upon TCR ligation, members of the Src family kinases Lck and Fyn phosphorylate the immunoreceptor tyrosine-based signalling motifs (ITAMs) located within the TCR-associated CD3 and ζ chains. This event results in the recruitment of the tyrosine kinase ζ chain–associated protein kinase of 70 kDa (ZAP-70) to the receptor. ZAP-70 is in turn activated and further phosphorylates the linker for activation of T cells (LAT), a transmembrane adaptor molecule that further assembles a complex leading to Ca2+

flux, Ras and protein kinase C (PKC) activation (Figure 1). These events ultimately culminate in gene transcription, proliferation and differentiation of T cells.

T-cell activation and differentiation depends on APCs such as dendritic cells (DCs), macrophages and B cells. Among them, DCs are highly specialized in antigen presentation and in T-cell priming [6]. DCs act as sentinels in the body where they capture antigens. Danger signals such as microbial products or cytokines from injured tissue activate DCs, which in turn migrate to secondary lymphoid organs, where they allow initiation of the immune response [7]. The nature of the stimulus dictates which kind of immune response will be set in motion [8]. Therefore, 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))

In the process of antigen presentation by DCs, macrophages or B cells, T cell activation is impaired.

**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

**Applicability domain**

**Evidence for perturbation of this molecular initiating event by stressor**
RelB deficient mice had an impaired cellular immunity, as observed in contact sensitivity reaction (Weih et al., 1995).

References

**KE: Title: Inhibition, Impaired Ab production**

**Short name:** Inhibition, Impaired Ab production

**Biological organization: Molecular**

**Cell term: B cell**

**Organ term: immune system**

**Stressors:**

**Key event description**

ACTIVATION OF B CELLS
Initial encounter of antigen by B-cells occurs in peripheral lymphoid organ where free antigens gain access via lymphatics or are carried by homing dendritic cells, professional antigen-presenting cells from peripheral tissues. The B-cell receptor on B-lymphocytes efficiently captures antigen which is then internalized,processed and returned to the cell surface as peptides bound to Class II MHC molecules. Antigenactivated B-cells then migrate toward the T-cell zones of the lymphoid tissue. Humoral response to most protein antigens requires help from CD4+ T-cells. B-cell–T-cell interaction leads to activation, proliferation and further differentiation of B-cells into plasma cells. Some B-cells migrate from the T-cell zone into a nearby lymphoid follicle where they proliferate and differentiate and establish secondary germinal centers.The rapid proliferation of cells in the germinal centre greatly increases the number of B-cells specific for the pathogen that initiated the antibody response. Furthermore, in the germinal center, somatic hypermutation of immunoglobulin-variable domain genes and affinity maturation occur such that there is a switch from IgM to other isotypes of antibodies and increase in the affinity of antibodies for the inducing antigen. These antigen-activated B-cells then come into contact with specialized stromal cells called follicular dendritic cells that bear unprocessed antigens trapped within the lymphoid follicles. These cells provide survival signals for mature B-lymphocytes that bind cognate antigen on their surface with high affinity. Those B-cells that fail to bind die by apoptosis. Thus, those B-cells that have high-affinity binding to antigens survive the selection process, leave the germinal center to become either memory B-cells or antibody-secreting plasma cells. Plasma cells migrate to the bone marrow and produce the majority of circulating immunoglobins. B-cells that become

memory B-cells reside in the lymphoid organ and can be rapidly activated upon subsequent challenge with the same antigen.

B-CELL–T-CELL INTERACTION

Helper T-cells which recognize antigen on the surface of B-cells become activated and synthesize both cell bound and secreted effector molecules that synergize in B-cell activation (Fig. 1). CD40 ligand (CD40L) is expressed on activated helper T-cells, that binds to CD40 on B-cell surface. Antigen binding and CD40–CD40L interaction provide signals that drive B-cell activation, proliferation and differentiation into plasma cells. Activated B-cells also express other co-stimulatory molecules such as surface B7.1 and B7.2 proteins that bind to CD28 on the surface of T-cells to enhance cognate interaction as well as driving T-cell activation. The B7 molecules are members of the immunoglobulin superfamily that bind to CD28 on naı¨ve T-cells and an additional receptor, CTLA-4 that is expressed on activated T-cells. CTLA-4 binds B7 molecules with higher avidity than CD28 and transduces a negative signal to the activated T-cells in order to limit excessive proliferative response of these activated T-cells. Soluble factors like cytokines are also important inducers of B-cell activation. Interleukin (IL)-4 preferentially induce switching of immunoglobulin isotype to IgG1 and IgE, whereas tissue growth factor (TGF)-b induces switching to IgG2b and IgA. Interferon (IFN)-c induces IgG2a and IgG3 production by activated B-lymphocytes.(reviewed by Mok (Mok, 2010)).

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.

**How it is measured or detected.**

Ab production can be measured by ELISA.

**Applicability domain**

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

Mice lacking the p50 subunit of NF-KB show no developmental abnormalities, but exhibit multifocal defects in immune responses involving B lymphocytes and nonspecific responses to infection. B cells do not proliferate in response to bacterial lipopolysaccharide and are defective in basal and specific antibody production. Mice lacking p50 are unable effectively to clear L. monocytogenes and are more susceptible

to infection with S. pneumoniae (Sha et al., 1995).

References

**AO: Title: Increased susceptibility to infection**

**Short name:** Increased susceptibility to infection

**Biological organization: Individual**

**Key event description**

Complications from infection as a side-effect of administering FK506 was found to be similar to that of cyclosporin A (Ekberg et al. 2007), and recipients of liver transplants treated with FK506 were found to have suffered bacterial, viral, and fungal infections (Alessiani et al. 1991, Fung et al. 1991).

Defect of IL-1 signaling caused by knockout of mice gene or administration of IL-1 receptor antagonist or neutralizing antibodies to human leads to the increased susceptibility to infection. Moreover, polymorphism of IL-1b or IL-1Ra leads to the increased susceptibility to tuberculosis or fungal infection.

**Definition**

Increased incidence of bacterial infection, tuberculous infection, and viral infection in humans and mice.



**How it is measured or detected.**

By comparison of the incidence of infection between individuals exposed to stressors and non-exposed individuals.

**Applicability domain**

The increased susceptibiity to infection caused by IL-1RA or anti-IL-1 antibody has been reported in both humans and mice.

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

**Regulatory significance of the adverse outcome**

References

**Key Event Relationship**

**Blocking of IL-1R leads to impaired IL-1 signaling**

**Key Event Relationship Description**

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. Through conserved cytosolic regions called Toll- and IL-1R–like (TIR) domains, 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. Therefore, the suppression of the binding of IL-1 to IL-1R1 suppresses the recruitment of IL-1RacP, which results in impaired IL-1 signaling.

Evidence Supporting this KER

**Biological plausibility**

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-R like rilonacept. The binding of IL-1β to IL-1R1 can also be inhibited by anti-IL-1β antibody (anti-IL-1β antibody).

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; Migkos et al., 2015). 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 increased

susceptibility to infection (De Benedetti et al., 2018; Imagawa et al., 2013; Lachmann et al., 2009; Schlesinger et al., 2012).

**Empirical Evidence**

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

1) Down modulation of EGF receptor (3 nM of ED50) (Dripps et al., 1991)
2) Suppression of IL-1-induced endothelial cell-leukocyte adhesion (approximately 10 ng/ml of ED50) (Dripps et al., 1991)
3) rhIL-1a-induced mouse thymocytes proliferation (ED50 almost 3 μg/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-lra infusion synthesized significantly less interleukin 6 ex vivo than PBMC from saline-injected controls. (Granowitz et al., 1992)

**Canakinumab (ACZ885, Ilaris):**

Four patients with active disease each received an i.v. dose of 10 mg/kg canakinumab. relapse. This was possible because IL-1b, which was undetectable in sera of patients at baseline (assay detection limit <0.1 pg/ml), could be detected by an assay that measured IL-1b complexed with antibody. (Lachmann et al. 2009)

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 (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).

**Uncertainties and Inconsistencies**

**Quantitative Understanding of the Linkage**

**Response-response relationship**

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

IL-lra alone at concentrations as high as 1 μg/mL did not induce IL-la, IL-lb, 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-lb reduced IL-lb-induced IL-la by 95% (P = .01) and IL-la-induced IL-1b by 73% (P < .01). In elutriated monocytes, a 10-fold molar excess of IL-lra reduced IL-lb-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).

**Time-scale**
**Known modulating factors**
**Known Feedforward/Feedback loops influencing this KER**
**Domain of Applicability**

**Decreased IL-1 production leads to impaired IL-1 signaling**

**Key Event Relationship Description**

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. Through conserved cytosolic regions called Toll- and IL-1R–like (TIR) domains, 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.

**Evidence Supporting this KER**

**Biological plausibility**

**Empirical Evidence**

**Uncertainties and Inconsistencies**

**Quantitative Understanding of the Linkage**

**Response-response relationship**

**Time-scale**

**Known modulating factors**

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

**Domain of Applicability**

**Impaired IL-1 signaling leads to inhibition, Nuclear facto kappa B (NF-kB)**

**Key Event Relationship Description**

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). Mice lacking MYD88 or IRAK4 show severe defects in IL-1 signaling (Adachi et al., 1998; Medzhitov et al., 1998; Suzuki et al., 2002). Similarly, humans with mutations in the IRAK4 gene have defects in IL-1RI and Toll-like receptor (TLR) signaling (Picard et al., 2003). 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. (Brikos et al., 2007).

**Evidence Supporting this KER**

**Biological plausibility**

**Empirical Evidence**

**Uncertainties and Inconsistencies**

**Quantitative Understanding of the Linkage**

**Response-response relationship**

**Time-scale**

**Known modulating factors**

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

**Domain of Applicability**

**Inhibition, Nuclear facto kappa B (NF-κB) leads to impaired T cell activation**
**Key Event Relationship Description**

The general consensus understanding is that 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). Phosphory- lated CD3 activates the T cell specific tyrosine kinase, zeta-chain associated protein kinase (ZAP-70), which phosphorylates the adapter proteins linker for activation of T cells (LAT) and SH2 domain containing leukocyte protein of 76 kDa (SLP-76), causing SLP-76 to bind to VAV1. The VAV1–SLP76–IL-2-inducible T cell kinase (ITK) complex activates phospholipase (PL)C*g*1, generat- ing inositol 1,4,5-tripohosphate (IP3) and diacylglycerol (DAG), which ultimately trigger calcium release and pro- tein kinase (PK)C activation, respectively. Activation of a specific PKC isoform, PKC*u*, connects the above described TCR proximal signaling events to distal events that ulti- mately lead to NF-*k*B activation. Importantly, PKC*u* activation is also driven by engagement of the T cell co-stimulatory receptor CD28 by B7 ligands on antigen- presenting cells (APCs). This molecular interaction activates phosphoinositide 3-kinase (PI3K), inducing triggers a conformational change, causing CARMA1 to bind to B cell leukemia/lymphoma (BCL10) and mucosa- associated lymphoid tissue lymphoma translocation pro- tein (MALT1), forming the CARMA1-BCL10-MALT1 (CBM) complex. Through a mechanism that may involve TNF receptor-associated factor (TRAF6), both BCL10 and MALT1 become polyubiquitinated. The I*k*B kinase (IKK) complex is then recruited to the CBM complex via the IKK*g* polyubiquitin binding motif. This association leads to polyubiquitination of IKK*g* and phosphorylation of IKK*b* by TGF-*b* activated kinase (TAK1), activating IKK*b*. IKK*b* then phosphorylates inhibitor of *k*B (I*k*B*a*), triggering its proteasomal degradation, enabling nuclear translocation of canonical NF-*k*B heterodimers comprised of p65 reticuloendotheliosis viral oncogene homolog A (RELA) and p50 proteins. Once in the nucleus, NF-*k*B governs the transcription of numerous genes involved in T cell survival, proliferation, and effector functions (Paul and Schaefer, 2013).

**Evidence Supporting this KER**

**Biological plausibility**

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

**Empirical Evidence**

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 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).

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).

In Balb/c mice (p.o.) treated with naringin (20, 40 and 80 mg/kg) for 14 days, compared with the vehicle-treated and arthritic-control mice, the naringin treatment demonstrated a considerable decrease in the level of T cells, CD4+GITR+, Th1 cytokine and inflammatory mediator expressions. In contrast, naringin treatment resulted in significantly up-regulated Treg and Th2 cytokine levels (Ahmad et al., 2014).

**Uncertainties and Inconsistencies**

**Quantitative Understanding of the Linkage**

**Response-response relationship**

**Time-scale**

**Known modulating factors**

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

**Domain of Applicability**

**Impaired T cell activation to Impaired Ab production**

**Key Event Relationship Description**

'Help' to B cells is not a single product of TFH cells and not even a single process. T cell help to B cells can be divided into seven distinct functions, proliferation, survival, plasma cell differentiation, somatic hypermutation, class-switch recombination, adhesion and attraction. These seven different forms of help are all contributors to $T_{FH}$ cell–B cell interactions, and each process consists of multiple pathways. Furthermore, some molecules have a role in several different forms of help.

The simplest B cell help function that is provided by $T_{FH}$ cells is the induction of B cell proliferation. CD40L is the most prominent protein expressed by $T_{FH}$ cells that contributes to pro-mitotic signalling in B cells64. Survival signals from TFH cells are also crucial, as germinal centre B cells are exquisitely pro-apoptotic. IL-4 produced by $T_{FH}$ cells triggers pro-survival signals to germinal centre B cells via the IL-4 receptor complex. Somatic hypermutation is central to germinal centre biology and the primary purpose of germinal centres is to facilitate affinity maturation of B cells via sequential rounds of immunoglobulin gene mutation and selection. The enzyme activation-induced cytidine deaminase (AID) induces the DNA damage in the immunoglobulin genes that is then converted into mutations by DNA repair enzymes. BCL-6 must be co-expressed with AID by the germinal centre B cell to repress the DNA damage response programme that would otherwise trigger self-destruction of the cell. The signals that induce AID and BCL-6 expression by B cells are not entirely defined, but CD40L, IL-4 and IL-21 contribute. Indeed, the combination of CD40L, IL-4 and IL-21 in different ratios seems to be the primary mix of T cell help signals that control B cell proliferation, somatic hypermutation and differentiation. Class-switch recombination can also be induced by instructive signals from $T_{FH}$ cells to B cells. AID is necessary for class-switch recombination, but the specific target of the heavy chain constant region gene recombination depends on additional factors that are selectively activated by different cytokines, which predominantly, but not exclusively, come from CD4+ T cells. Human IgM to IgG class-switch recombination is most efficiently induced by IL-21, whereas IgE recombination is induced by a high IL-4 to IL-21 ratio.

B cell help crucially depends on cell contact, probably because of a mixture of cell-surface co-stimulatory ligand interactions and directional cytokine production during cognate interactions. Therefore, adhesion molecules expressed by $T_{FH}$ cells and B cells are necessary components of T cell help to B cells, as they regulate the overall duration of the 'pas de deux'. The most dramatic example of this requirement is SAP, which is

described above. SLAM-associated protein (SAP; also known as SH2D1A) binds to the intracellular domains of SLAM family surface receptors, which are involved in cell–cell adhesion. In the absence of SAP, the duration of B cell–T cell adhesion is short and inadequate for the $T_{FH}$ cell to provide sufficient help signals to the B cell. This leads to a general defect in SAP-dependent T cell help to B cells and thus a loss of antigen-specific B cell proliferation and survival, as well as a complete loss of germinal centres and of most memory B cells and long-lived plasma cells.
Finally, chemoattraction is another component of T cell help to B cells. CXC-chemokine ligand 13 (CXCL13) is the ligand for CXCR5 and human germinal centre TFH cells constitutively secrete copious quantities of CXCL13, which probably recruits B cells to colocalize with the TFH cells and to facilitate confinement of the B cells to the germinal centre. Notably, CXCL13 signalling via CXCR5 also modifies B cell adhesion and lymphotoxin synthesis, which shows that CXCL13 also has cytokine-type functions. Thus, chemoattraction is another form of T cell help to B cells.
Therefore, it is conceivable that impaired T cell activation leads to impaired B cell activation and antibody production.

**Evidence Supporting this KER**
**Biological plausibility**
**Empirical Evidence**
**Uncertainties and Inconsistencies**
**Quantitative Understanding of the Linkage**
**Response-response relationship**
**Time-scale**
**Known modulating factors**
**Known Feedforward/Feedback loops influencing this KER**
**Domain of Applicability**

**Impaired T cell activation and Ab production to increased susceptibility to infection**

**Key Event Relationship Description**

Normal T cell and B cell function is indispensable for host defense mechanism.

**Evidence Supporting this KER**

**Biological plausibility**

SCID mice and patients with severe combined immunodeficiency are extremely susceptible to bacterial and viral infection.

**Empirical Evidence**

**Uncertainties and Inconsistencies**

**Quantitative Understanding of the Linkage**

**Response-response relationship**

**Time-scale**

**Known modulating factors**

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

**Domain of Applicability**

## References


Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., Akira, S., 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity 9, 143-150.

Ahmad, S.F., Zoheir, K.M., Abdel-Hamied, H.E., Ashour, A.E., Bakheet, S.A., Attia, S.M., Abd-Allah, A.R., 2014. Amelioration of autoimmune arthritis by naringin through modulation of T regulatory cells and Th1/Th2 cytokines. Cellular immunology 287, 112-120.

Alten, R., Gram, H., Joosten, L.A., van den Berg, W.B., Sieper, J., Wassenberg, S., Burmester, G., van Riel, P., Diaz-Lorente, M., Bruin, G.J., Woodworth, T.G., Rordorf, C., Batard, Y., Wright, A.M., Jung, T., 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 research & therapy 10, R67.

Arend, W.P., Welgus, H.G., Thompson, R.C., Eisenberg, S.P., 1990. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. The Journal of clinical investigation 85, 1694-1697.

Brikos, C., Wait, R., Begum, S., O'Neill, L.A., Saklatvala, J., 2007. Mass spectrometric analysis of the endogenous type I interleukin-1 (IL-1) receptor signaling complex formed after IL-1 binding identifies IL-1RAcP, MyD88, and IRAK-4 as the stable components. Molecular & cellular proteomics : MCP 6, 1551-1559.

Cavalli, G., Franchini, S., Aiello, P., Guglielmi, B., Berti, A., Campochiaro, C., Sabbadini, M.G., Baldissera, E., Dagna, L., 2015. Efficacy and safety of biological agents in adult-onset Still's disease. Scandinavian journal of rheumatology 44, 309-314.

Chaudhary, L.R., Avioli, L.V., 1994. Dexamethasone regulates IL-1 beta and TNF-alpha-induced interleukin-8 production in human bone marrow stromal and osteoblast-like cells. Calcified tissue international 55, 16-20.

Church, L.D., McDermott, M.F., 2009. Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. Current opinion in molecular therapeutics 11, 81-89.

De Benedetti, F., Gattorno, M., Anton, J., Ben-Chetrit, E., Frenkel, J., Hoffman, H.M., Kone-Paut, I., Lachmann, H.J., Ozen, S., Simon, A., Zeft, A., Calvo Penades, I., Moutschen, M., Quartier, P., Kasapcopur, O., Shcherbina, A., Hofer, M.,

Hashkes, P.J., Van der Hilst, J., Hara, R., Bujan-Rivas, S., Constantin, T., Gul, A., Livneh, A., Brogan, P., Cattalini, M., Obici, L., Lheritier, K., Speziale, A., Junge, G., 2018. Canakinumab for the Treatment of Autoinflammatory Recurrent Fever Syndromes. The New England journal of medicine 378, 1908-1919.

Dhimolea, E., 2010. Canakinumab. mAbs 2, 3-13.

Dinarello, C.A., 2018. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunological reviews 281, 8-27.

Dripps, D.J., Brandhuber, B.J., Thompson, R.C., Eisenberg, S.P., 1991. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. The Journal of biological chemistry 266, 10331-10336.

Economides, A.N., Carpenter, L.R., Rudge, J.S., Wong, V., Koehler-Stec, E.M., Hartnett, C., Pyles, E.A., Xu, X., Daly, T.J., Young, M.R., Fandl, J.P., Lee, F., Carver, S., McNay, J., Bailey, K., Ramakanth, S., Hutabarat, R., Huang, T.T., Radziejewski, C., Yancopoulos, G.D., Stahl, N., 2003. Cytokine traps: multi-component, high-affinity blockers of cytokine action. Nature medicine 9, 47-52.

Fenini, G., Contassot, E., French, L.E., 2017. Potential of IL-1, IL-18 and Inflammasome Inhibition for the Treatment of Inflammatory Skin Diseases. Frontiers in pharmacology 8, 278.

Finch-Arietta, M.B., Cochran, F.R., 1991. Cytokine production in whole blood ex vivo. Agents and actions 34, 49-52.

Fleischmann, R.M., Schechtman, J., Bennett, R., Handel, M.L., Burmester, G.R., Tesser, J., Modafferi, D., Poulakos, J., Sun, G., 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 and rheumatism 48, 927-934.

Fremond, C.M., Yeremeev, V., Nicolle, D.M., Jacobs, M., Quesniaux, V.F., Ryffel, B., 2004. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. The Journal of clinical investigation 114, 1790-1799.

Gabay, C., Lamacchia, C., Palmer, G., 2010. IL-1 pathways in inflammation and human diseases. Nature reviews. Rheumatology 6, 232-241.

Genovese, M.C., Cohen, S., Moreland, L., Lium, D., Robbins, S., Newmark, R., Bekker, P., 2004. Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. Arthritis and rheumatism 50, 1412-1419.

Granowitz, E.V., Clark, B.D., Vannier, E., Callahan, M.V., Dinarello, C.A., 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., Johansen, P., Brombacher, F., Bachmann, M.F., 2011. Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. Vaccine 29, 1339-1346.

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

Horino, T., Matsumoto, T., Ishikawa, H., Kimura, S., Uramatsu, M., Tanabe, M., Tateda, K., Miyazaki, S., Aramaki, Y., Iwakura, Y., Yoshida, M., Onodera, S., Yamaguchi, K., 2009. Interleukin-1 deficiency in combination with macrophage depletion increases susceptibility to Pseudomonas aeruginosa bacteremia. Microbiology and immunology 53, 502-511.

Huang, H., Wang, Y., 2017. The protective effect of cinnamaldehyde on lipopolysaccharide induced acute lung injury in mice. Cellular and molecular biology (Noisy-le-Grand, France) 63, 58-63.

Imagawa, T., Nishikomori, R., Takada, H., Takeshita, S., Patel, N., Kim, D., Lheritier, K., Heike, T., Hara, T., Yokota, S., 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). Clinical and experimental rheumatology 31, 302-309.

Jeon, Y.J., Han, S.H., Lee, Y.W., Lee, M., Yang, K.H., Kim, H.M., 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.

Juffermans, N.P., Florquin, S., Camoglio, L., Verbon, A., Kolk, A.H., Speelman, P., van Deventer, S.J., van Der Poll, T., 2000. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. The Journal of infectious diseases 182, 902-908.

Kapur, S., Bonk, M.E., 2009. Rilonacept (arcalyst), an interleukin-1 trap for the treatment of cryopyrin-associated periodic syndromes. P & T : a peer-reviewed journal for formulary management 34, 138-141.

Karpenko, M.N., Vasilishina, A.A., Gromova, E.A., Muruzheva, Z.M., Bernadotte, A., 2018. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-6,

interleukin-10, and tumor necrosis factor-alpha levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. Cellular immunology 327, 77-82.

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nature reviews. Immunology 16, 626-638.

Kullenberg, T., Lofqvist, M., Leinonen, M., Goldbach-Mansky, R., Olivecrona, H., 2016. Long-term safety profile of anakinra in patients with severe cryopyrin-associated periodic syndromes. Rheumatology (Oxford, England) 55, 1499-1506.

Lachmann, H.J., Kone-Paut, I., Kuemmerle-Deschner, J.B., Leslie, K.S., Hachulla, E., Quartier, P., Gitton, X., Widmer, A., Patel, N., Hawkins, P.N., 2009. Use of canakinumab in the cryopyrin-associated periodic syndrome. The New England journal of medicine 360, 2416-2425.

Lequerre, T., Quartier, P., Rosellini, D., Alaoui, F., De Bandt, M., Mejjad, O., Kone-Paut, I., Michel, M., Dernis, E., Khellaf, M., Limal, N., Job-Deslandre, C., Fautrel, B., Le Loet, X., Sibilia, J., 2008. Interleukin-1 receptor antagonist (anakinra) treatment in patients with systemic-onset juvenile idiopathic arthritis or adult onset Still disease: preliminary experience in France. Annals of the rheumatic diseases 67, 302-308.

Li, W.D., Ran, G.X., Teng, H.L., Lin, Z.B., 2002. Dynamic effects of leflunomide on IL-1, IL-6, and TNF-alpha activity produced from peritoneal macrophages in adjuvant arthritis rats. Acta pharmacologica Sinica 23, 752-756.

McIntyre, K.W., Stepan, G.J., Kolinsky, K.D., Benjamin, W.R., Plocinski, J.M., Kaffka, K.L., Campen, C.A., Chizzonite, R.A., Kilian, P.L., 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. The Journal of experimental medicine 173, 931-939.

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.

Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S., Janeway, C.A., Jr., 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 2, 253-258.

Migkos, M.P., Somarakis, G.A., Markatseli, T.E., Matthaiou, M., Kosta, P., Voulgari, P.V., Drosos, A.A., 2015. Tuberculous pyomyositis in a rheumatoid arthritis

patient treated with anakinra. Clinical and experimental rheumatology 33, 734-736.

Mok, M.Y., 2010. The immunological basis of B-cell therapy in systemic lupus erythematosus. Int J Rheum Dis 13, 3-11.

Monick, M.M., Aksamit, T.R., Geist, L.J., Hunninghake, G.W., 1994. Dexamethasone inhibits IL-1 and TNF activity in human lung fibroblasts without affecting IL-1 or TNF receptors. The American journal of physiology 267, L33-38.

Morand, E.F., Rickard, D., Goulding, N.J., 1993. Lack of involvement of lipocortin 1 in dexamethasone suppression of IL-1 release. Mediators of inflammation 2, 49-52.

Newman, S.P., Flower, R.J., Croxtall, J.D., 1994. Dexamethasone suppression of IL-1 beta-induced cyclooxygenase 2 expression is not mediated by lipocortin-1 in A549 cells. Biochemical and biophysical research communications 202, 931-939.

Nishioka, C., Ikezoe, T., Jing, Y., Umezawa, K., Yokoyama, A., 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.

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. International archives of allergy and immunology 176, 91-100.

Orciuolo, E., Galimberti, S., Petrini, M., 2007. Bortezomib inhibits T-cell function versus infective antigenic stimuli in a dose-dependent manner in vitro. Leukemia research 31, 1026-1027.

Paul, S., Schaefer, B.C., 2013. A new look at T cell receptor signaling to nuclear factor-kappaB. Trends in immunology 34, 269-281.

Pernis, A.B., 2007. Estrogen and CD4+ T cells. Curr Opin Rheumatol 19, 414-420.

Picard, C., Puel, A., Bonnet, M., Ku, C.L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J., Fieschi, C., Elbim, C., Hitchcock, R., Lammas, D., Davies, G., Al-Ghonaium, A., Al-Rayes, H., Al-Jumaah, S., Al-Hajjar, S., Al-Mohsen, I.Z., Frayha, H.H., Rucker, R., Hawn, T.R., Aderem, A., Tufenkeji, H., Haraguchi, S., Day, N.K., Good, R.A., Gougerot-Pocidalo, M.A., Ozinsky, A., Casanova, J.L., 2003. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science (New York, N.Y.) 299, 2076-2079.

Picard, C., von Bernuth, H., Ghandil, P., Chrabieh, M., Levy, O., Arkwright, P.D., McDonald, D., Geha, R.S., Takada, H., Krause, J.C., Creech, C.B., Ku, C.L., Ehl, S., Marodi, L., Al-Muhsen, S., Al-Hajjar, S., Al-Ghonaium, A., Day-Good, N.K., Holland, S.M., Gallin, J.I., Chapel, H., Speert, D.P., Rodriguez-Gallego, C., Colino, E., Garty, B.Z., Roifman, C., Hara, T., Yoshikawa, H., Nonoyama, S., Domachowske, J., Issekutz, A.C., Tang, M., Smart, J., Zitnik, S.E., Hoarau, C., Kumararatne, D.S., Thrasher, A.J., Davies, E.G., Bethune, C., Sirvent, N., de Ricaud, D., Camcioglu, Y., Vasconcelos, J., Guedes, M., Vitor, A.B., Rodrigo, C., Almazan, F., Mendez, M., Arostegui, J.I., Alsina, L., Fortuny, C., Reichenbach, J., Verbsky, J.W., Bossuyt, X., Doffinger, R., Abel, L., Puel, A., Casanova, J.L., 2010. Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency. Medicine 89, 403-425.

Pordanjani, S.M., Hosseinimehr, S.J., 2016. The Role of NF-kB Inhibitors in Cell Response to Radiation. Current medicinal chemistry 23, 3951-3963.

Qiu, H.B., Pan, J.Q., Zhao, Y.Q., Chen, D.C., 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 = Acta pharmacologica Sinica 18, 165-168.

Quartier, P., 2011. Interleukin-1 antagonists in the treatment of autoinflammatory syndromes, including cryopyrin-associated periodic syndrome. Open access rheumatology : research and reviews 3, 9-18.

Radons, J., Dove, S., Neumann, D., Altmann, R., Botzki, A., Martin, M.U., Falk, W., 2003. The interleukin 1 (IL-1) receptor accessory protein Toll/IL-1 receptor domain: analysis of putative interaction sites in vitro mutagenesis and molecular modeling. The Journal of biological chemistry 278, 49145-49153.

Ravizza, T., Lucas, S.M., Balosso, S., Bernardino, L., Ku, G., Noe, F., Malva, J., Randle, J.C., Allan, S., Vezzani, A., 2006. Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. Epilepsia 47, 1160-1168.

Satou, Y., Nosaka, K., Koya, Y., Yasunaga, J.I., Toyokuni, S., Matsuoka, M., 2004. Proteasome inhibitor, bortezomib, potently inhibits the growth of adult T-cell leukemia cells both in vivo and in vitro. Leukemia 18, 1357-1363.

Scanga, C.A., Bafica, A., Feng, C.G., Cheever, A.W., Hieny, S., Sher, A., 2004. MyD88-deficient mice display a profound loss in resistance to Mycobacterium tuberculosis associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. Infection and immunity 72, 2400-2404.

Schlesinger, N., Alten, R.E., Bardin, T., Schumacher, H.R., Bloch, M., Gimona, A., Krammer, G., Murphy, V., Richard, D., So, A.K., 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. Annals of the rheumatic diseases 71, 1839-1848.

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

Shuck, M.E., Eessalu, T.E., Tracey, D.E., Bienkowski, M.J., 1991. Cloning, heterologous expression and characterization of murine interleukin 1 receptor antagonist protein. European journal of immunology 21, 2775-2780.

Simeoni, L., Thurm, C., Kritikos, A., Linkermann, A., 2016. Redox homeostasis, T cells and kidney diseases: three faces in the dark. Clin Kidney J 9, 1-10.

Stack, J.H., Beaumont, K., Larsen, P.D., Straley, K.S., Henkel, G.W., Randle, J.C., Hoffman, H.M., 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. Journal of immunology (Baltimore, Md. : 1950) 175, 2630-2634.

Straub, R.H., 2007. The complex role of estrogens in inflammation. Endocr Rev 28, 521-574.

Suzuki, N., Suzuki, S., Duncan, G.S., Millar, D.G., Wada, T., Mirtsos, C., Takada, H., Wakeham, A., Itie, A., Li, S., Penninger, J.M., Wesche, H., Ohashi, P.S., Mak, T.W., Yeh, W.C., 2002. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature 416, 750-756.

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.

van Furth, A.M., Seijmonsbergen, E.M., Langermans, J.A., van der Meide, P.H., van Furth, R., 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. Clinical and diagnostic laboratory immunology 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.

von Bernuth, H., Picard, C., Jin, Z., Pankla, R., Xiao, H., Ku, C.L., Chrabieh, M., Mustapha, I.B., Ghandil, P., Camcioglu, Y., Vasconcelos, J., Sirvent, N., Guedes, M., Vitor, A.B., Herrero-Mata, M.J., Arostegui, J.I., Rodrigo, C., Alsina, L., Ruiz-Ortiz, E., Juan, M., Fortuny, C., Yague, J., Anton, J., Pascal, M., Chang, H.H., Janniere, L., Rose, Y., Garty, B.Z., Chapel, H., Issekutz, A., Marodi, L., Rodriguez-Gallego, C., Banchereau, J., Abel, L., Li, X., Chaussabel, D., Puel, A., Casanova, J.L., 2008. Pyogenic bacterial infections in humans with MyD88 deficiency. Science (New York, N.Y.) 321, 691-696.

Wang, A.L., Yu, A.C., Lau, L.T., Lee, C., Wu le, M., Zhu, X., Tso, M.O., 2005. Minocycline inhibits LPS-induced retinal microglia activation. Neurochemistry international 47, 152-158.

Wang, Y., Sun, B., Volk, H.D., Proesch, S., Kern, F., 2011. Comparative study of the influence of proteasome inhibitor MG132 and ganciclovir on the cytomegalovirus-specific CD8(+) T-cell immune response. Viral immunology 24, 455-461.

Weber, A., Wasiliew, P., Kracht, M., 2010a. Interleukin-1 (IL-1) pathway. Sci Signal 3, cm1.

Weber, A., Wasiliew, P., Kracht, M., 2010b. Interleukin-1beta (IL-1beta) processing pathway. Sci Signal 3, cm2.

Weber, A.N.R., Cardona Gloria, Y., Cinar, O., Reinhardt, H.C., Pezzutto, A., Wolz, O.O., 2018. Oncogenic MYD88 mutations in lymphoma: novel insights and therapeutic possibilities. Cancer immunology, immunotherapy : CII 67, 1797-1807.

Weih, F., Carrasco, D., Durham, S.K., Barton, D.S., Rizzo, C.A., Ryseck, R.P., Lira, S.A., Bravo, R., 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.

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.

Xu, F., Wang, F., Wen, T., Sang, W., Wang, D., Zeng, N., 2017. Inhibition of NLRP3 inflammasome: a new protective mechanism of cinnamaldehyde in endotoxin poisoning of mice. Immunopharmacology and immunotoxicology 39, 296-304.

Yamada, H., Mizumo, S., Horai, R., Iwakura, Y., Sugawara, I., 2000. Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. Laboratory investigation; a journal of technical methods and pathology 80, 759-767.

Yokota, S., Imagawa, T., Nishikomori, R., Takada, H., Abrams, K., Lheritier, K., Heike, T., Hara, T., 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. Clinical and experimental rheumatology 35 Suppl 108, 19-26.

Zhao, J., Zhang, X., Dong, L., Wen, Y., Zheng, X., Zhang, C., Chen, R., Zhang, Y., Li, Y., He, T., Zhu, X., Li, L., 2015. Cinnamaldehyde inhibits inflammation and brain damage in a mouse model of permanent cerebral ischaemia. British journal of pharmacology 172, 5009-5023.