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

令和元年度 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試験を実施した。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有害性発現経路（AOP: Adverse Outcome Pathway）の作成が不可欠である。テスト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、IL-1シグナルの欠損により易感染性を生じるというAOPを作成し9月に提出した。OECD EAGMSTの内部レビューアーの指摘を受け、タイトル、Molecular Initiating Event (MIE)の構成を変更し、またQuantitative Understanding, Empirical supportに関する論文を再調査し3月に再提出した。その結果、AOP : Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (Aop:277)は外部評価へと移行した。

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

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。昨年度，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，MITA の 4 種類のパラメーターの内の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 を施行したところ，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。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 試験を終了した。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

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，昨年度に引き続きIL-1シグナルの欠損により易感染性を生じるというAOPを作成した(Aop:277)。

C.2. AOP WIKI への登録

作成した AOP を Inhibition of IL-1 signaling という題名で 9 月に AOP-Wiki にアップロードした。(AOP：277)レビューアーより下記の指摘を受けこれらの修正を行い 3 月に再提出した。

- AOP のタイトルに Adverse Outcome を含めるようにとのコメントがありタイトルを Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection に変更した。

- abstract に weight of evidence などの記載も見られ冗長になっているとの指摘がありこれらの記載を他所に移し abstract を簡素化した。
- Molecular Initiating Event (MIE)は一つの AOP につき一つにするべきとの指摘がありMIE の構成を変更し MIE を一つとした。
- Key Events についても非専門家にも理解できるように記載するようにという指摘がありそのように修正した。
- Quantitative Understanding については適切な過去の論文が少ないことを記載し、関連を High から Moderate または Not Specified に変更した。

現時点での最新の AOP-Wiki への登録内容を Appendix 1 に示す。
その結果，AOP : Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection（Aop:277)は外部評価へと移行した。

D. 考察

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試験を終了した。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。
参照した過去の論文についてはノックアウトマウスを用いた実験や阻害剤を生体に投与した知見が中心であり Quantitative Understanding を裏付ける論文が少なく記載が困難であったが、レビューアーよりの提案もありその旨を記載し提出した。
empirical evidence については AOP に関する過去のいくつかの論文で表にまとめ提示しているが、それに倣い本 AOP でも表にまとめ提出した。

引用文献

1. Kimura, Y., Fujimura, C., Ito, Y., Takahashi, T., Terui, H., Aiba, S. 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, 2018; 92. 2043-2054.
2. 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. 1816-30.

E. 結論

AOP : Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection （Aop:277)を作成し，AOP-WIKIに登録した。その結果AOPは外部評価へと移行した。

F. 研究発表

F.1. 論文発表

1. Hidaka, T., Fujimura, T., Aiba, S Aryl hydrocarbon receptor modulates carcinogenesis and maintenance of skin cancers. Frot Med, 2019; 6. 194-
2. Kimura, Y., Yasuno, R., Watanabe, M., Kobayashi, M., Iwaki, T., Fujimura, C., Ohmiya, Y., Yamakage, K., Nakajima, Y., Kobayashi, M., Mashimo, N., Takagi, Y., Omori, T., Corsini, E., Germolec, D., Inoue, T., Rogen, E.L., Kojima, H., Aiba, S. An international validation study of the IL-2 Luc assay for evaluating the potential immunotoxic effects of chemicals on T cells and a proposal for reference data for immunotoxicchemicals. Toxicol In Vitro, 2020; in press.

F.2. 学会発表

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2. 木村 裕、安野理恵、渡美香、小林 美和子、岩城知子、藤村千鶴、近江谷克裕、山影康次、中島芳浩、真下奈々、高木佑実、大森 崇、小島 肇、相場節也 : Multi-ImmunoTox Assay (MITA)の予測性評価に必要な文献に基づく化学物質免疫毒性分類の試み 日本動物実験代替法学会第 32 回大会 つくば (2019.11)

G. 知的財産権の出願・登録状況

G.1. 特許取得

なし

Appendix 1

AOP ID and Title:

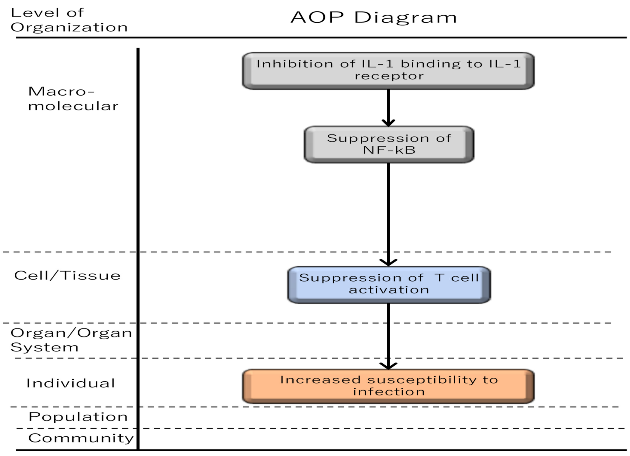
SNAPSHOT

Created at: 2020-03-31 06:49

AOP 277: Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection

Short Title: IL-1 inhibition

Graphical Representation



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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. 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-kB is already confirmed. In addition, the biological plausibility that suppressed NF-kB activation leads to impaired T cell activation 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. 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).

In this AOP, we considered inhibition of IL-1R activation as a MIE. The biological plausibility of the signaling cascade from the activation of IL-1R to the activation of NF-kB is already accepted. In addition, the biological plausibility that suppressed NF-kB 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.

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	Inhibition of IL-1 binding to IL-1 receptor (https://aopwiki.org/events/1700)	Inhibition of IL-1 binding to IL-1 receptor
2	KE	202	Inhibition, Nuclear factor kappa B (NF-kB) (https://aopwiki.org/events/202)	Inhibition, Nuclear factor kappa B (NF-kB)
3	KE	1702	Suppression of T cell activation (https://aopwiki.org/events/1702)	Suppression of T cell activation
4	AO	986	Increase, Increased susceptibility to infection (https://aopwiki.org/events/986)	Increase, Increased susceptibility to infection

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Inhibition of IL-1 binding to IL-1 receptor (https://aopwiki.org/relationships/2002)	adjacent	Inhibition, Nuclear factor kappa B (NF-kB)	High	Moderate
Inhibition, Nuclear factor kappa B (NF-kB) (https://aopwiki.org/relationships/2003)	adjacent	Suppression of T cell activation	High	Moderate
Suppression of T cell activation (https://aopwiki.org/relationships/2004)	adjacent	Increase, Increased susceptibility to infection	High	Not Specified

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

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 (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

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

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

216 organisms have orthologs with human gene RELB.

(https://www.ncbi.nlm.nih.gov/gene/5971 (https://www.ncbi.nlm.nih.gov/gene/5971))

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). Two IL-1 signaling antagonists, canakinumab (anti-IL-1b antibody) and rilonacept (soluble IL-1R) had been reported to increase 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 (Fremond et al., 2004; Picard et al., 2010; Scanga et al., 2004; von Bernuth et al., 2008). 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-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. 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).

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

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 et al., 2010a, b)

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

In T lineage cells, the temporal regulation of NF-kb 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-1b. (Gerondakis et al., 2014)

Suppression of T cell activation leads to Increase, Increased susceptibility to infection

First type immunity drives resistance to viruses and intracellular bacteria, such as Listeria monocytogenes, Salmonella spp. and Mycobacteria spp., as well as to intracellular protozoan parasites such as Leishmania spp. The T helper 1 signature cytokine interferon-γ has a central role in triggering cytotoxic mechanisms including macrophage polarization towards an antimicrobial response associated with the production of high levels of reactive oxygen species and reactive nitrogen species, activation of CD8 cytotoxic T lymphocytes and natural killer cells to kill infected cells via the perforin and/or granzyme B-dependent lytic pathway or via the ligation of surface death receptors; and B cell activation towards the production of cytolytic antibodies that target infected cells for complement and Fc receptor-mediated cellular cytotoxicity.

Resistance to extracellular metazoan parasites and other large parasites is mediated and/or involves second type immunity. Pathogen neutralization is achieved via different mechanisms controlled by T 2 signature cytokines, including interleukin-4, IL-5 and IL-13, and by additional type 2 cytokines such as thymic stromal lymphopoietin, IL-25 or IL-33, secreted by damaged cell. T 2 signature cytokines drive B cell activation towards the production of high-affinity pathogen-specific IgG1 and IgE antibodies that function via Fc-dependent mechanisms to trigger the activation of eosinophils, mast cells and basophils, expelling pathogens across epithelia.

T17 immunity confers resistance to extracellular bacteria such as Klebsiella pneumoniae, Escherichia coli, Citrobacter rodentium, Bordetella pertussis, Porphyromonas gingivalis and Streptococcus pneumoniae, and also to fungi such as Candida albicans, Coccidioides posadasii, Histoplasma capsulatum and Blastomyces dermatitidis. Activation of T 17 cells by cognate T cell receptor (TCR–MHC class II interactions and activation of group 3 innate lymphoid cells (ILC3s) via engagement of IL-1 receptor (IL-1R) by IL-1β secreted from damaged cells lead to the recruitment and activation of neutrophils. T 17 immunopathology is driven to a large extent by products of neutrophil activation, such as ROS and elastase (reviewed by Soares et al. (Soares et al., 2017).

Based on these evidences, the insufficient T cell or B cell function causes impaired resistance to infection.

Empirical support

This table summarizes the empirical support obtained from the experiment using several inhibitor or gene targeting mice.

concordance table empirical data				MIE	KE1	KE2
Reference	Chmical Initiator or deleted gene	dose	Species	Inhibition of IL-1 binding to IL-1 receptor	Inhibition, Nuclear factor kappa B (NF-kB)	Suppression of T cell activatior
Dripps et al. 1991	IL-1Ra (anakinra)			Equilibrium binding and kinetic experiments show that IL-1ra binds to the 80-kDa IL-1 receptor on the murine thymomcae ll line EL4 with an affinity (K_D = 150 pM) approximately equal to that of IL-la and IL-1b for this receptor		
Sigma-Aldrich Specification Sheet	IL-1Ra (anakinra)			Determined by its ability to inhibit the IL-1alpha stimulation of murine D10S cell. The expected ED50 is 20-40 ng/ml in the presence of 50 pg/ml of IL-1alpha.		
Fleischmann et al. 2003	IL-1Ra (anakinra)	100 mg of anakinra or placebo, administered daily by subcutaneous injection	human			
Genovese et al. 2004	IL-1Ra (anakinra)	treated with subcutaneous etanercept only (25 mg twice weekly), full-dosage etanercept (25 mg twice weekly) plus anakinra (100 mg/day), or half-dosage etanercept (25 mg once weekly) plus anakinra (100 mg/day) for 6 months	human			
Kullenberg et al. 2016	IL-1Ra (anakinra)	administered as daily s.c. injections	human			
Lequerre et al. 2008	IL-1Ra (anakinra)	treated with anakinra (1–2 mg/kg/day in children, 100 mg/day in adults)	human			
Migkos et al. 2015	IL-1Ra (anakinra)		human			
Settas et al. 2007	IL-1Ra (anakinra)		human			
Lee et al. 2004	IL-1Ra (anakinra)		intrathecal administration of IL-1ra (6 mg)		intrathecal pretreatment with IL-1ra (6 mg) or YVAD (0.5 mg) significantly inhibited NF-kB DNA-binding activity upregulation bilaterally (Fig. 3C). The intrathecal administration of IL-1ra or YVAD into non-inflamed animals produced no significant change in the DNA-binding activity of NF-kB p65.	
Vallejo et al. 2014	IL-1Ra (anakinra)	In diabetic rats treated with anakinra (100 or 160 mg/Kg/day for 3 or 7 days before sacrifice)	rat		In diabetic rats treated with anakinra (100 or 160 mg/Kg/day for 3 or 7 days before sacrifice) a partial improvement of diabetic endothelial dysfunction occurred, together with a reduction of vascular NADPH oxidase and NF-κB activation.	
Dhimolea et al. 2010	canakinumab			Canakinumab binds to human IL-1β with high affinity; the antibody-antigen dissociation equilibrium constant is approximately 35–40 pM. Cmax was 1.2, 1.2 and 1.5 pM for 1, 3 and 10 mg/kg antibody respectively, at days 42–56 after the first infusion.		
De Benedetti et al. 2018	canakinumab	150 mg subcutaneously every 4 weeks	human			
Imagawa et al. 2013	canakinumab	either 150 mg s.c. or 2 mg/kg for patients with a body weight ≤ 40 kg every 8 weeks for 24 weeks received	human			
Lachmann et al. 2009	canakinumab	150 mg of canakinumab subcutaneously every 8 weeks for up to 24 weeks	human			

Schlesinger et al. 2012	canakinumab	one dose of canakinumab 150 mg	human			
Textbook of Pediatric Rheumatology (Sixth Edition), 2011	rilonacept		human	Rilonacept has a very high binding affinity for IL-1 (dissociation constant ~1 pM), and it is specific for IL-1β and IL-1α.		
Hoffman et al. 2008	rilonacept	weekly subcutaneous injections (160 mg)	human			
Roell et al. 2010	gevokizumab (XOMA 052)		human		XOMA 052 neutralizes IL-1b stimulation of NFkB activation in HeLa cells stably expressing an NFkB-luciferase reporter construct with an IC$_{50}$ of ~1 pM at the EC$_{50}$ for this assay (25 pg/ml IL-1b).	
Mansouri et al. 2015	gevokizumab (XOMA 052)	receive gevokizumab 60 mg subcutaneously every 4 weeks for a total of three injections (12 weeks) with a 4-week follow-up period	human			
Issafras et al. 2014	gevokizumab (XOMA 052)		human (HeLa cells stably transfected with a nuclear factor-kB (NF-kB) luciferase reporter plasmid)		an average K$_B$ value (mean±S.D., n=3) of 4.8±4.4 pM	
Palombella et al. 1994	MG-132		human (in vitro)		Both MG115 and MG132 (at 20-40 mM) markedly inhibited the formation of p50 in HeLa S100 extracts (Figure 4A, lanes 8-13).	
Hellerbrand et al. 1998	MG-132		rat (in vitro)		ALLN (Fig. 3A) and MG132 (Fig. 3B) (10 mg/mL = 21mM) reduced the cytokine-mediated NFkB activation.	
Arlt et al. 2001	MG-132		human (in vitro)		In all cell lines, gliotoxin, MG132 (10 mM) or sulfasalazine strongly reduced VP16-induced NF-kB-driven luciferase expression.	
Ortiz-Lazareno et al. 2008	MG-132		human (in vitro)		The increase in NF-kB activation induced by LPS+PMA diminished significantly from 3.27-fold to 0.94-fold in the group treated with MG132(10 mM) and later stimulated with LPS+PMA (P < 0.002). The activation of NF-kB induced by LPS+PMA was blocked by MG132.	
Yu and Malek 2001	MG-132		mice (in vitro)			MG132 (50mM) stabilized IL-2 phosphorylated STAT5, which after 2 h in culture (Fig. 5A, lan
Wang et al. 2011	MG-132		human (in vitro)			CMV-specific cytotoxicity of CD decreased in the presence of M
Ohkusu-Tsukada et al. 2018	MG-132	repeatedly i.p. injected 200 nmol of MG132 on days 0, 3, 5, 7, 9, 11, 13, 15, 17, and 19.	mice (in vivo)			In vivo MG132 administration t DNFB-induced dermatitis reduc maintained the level of Th1 cel alleviation of dermatitis lesions serum IgE hyperproduction and
Satou et al. 2004	bortezomib		human (in vitro, in vivo)			potently inhibits the growth of a cells both in vivo and in vitro
Orciuolo et al. 2007	bortezomib	0.1 mM, 1 mM, 10 mM	human (in vitro)			the percentage of CD69/TNFa with the increment of bortezom
Matsumoto et al. 2005	dehydroxymethylepoxyquinomicin (DHMEQ)		human		The addition of DHMEQ (10 mg/mL) completely inhibited the activated NF-KB for at least 8 hours.	
Nishioka et al. 2008	dehydroxymethylepoxyquinomicin (DHMEQ)		human (in vitro)		DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF-kB in Jurkat cells via inhibition of degradation of IkBa.	Exposure of PBMC to PHA grea expression of IFN-g, IL-2 and TNF-a (Fig. these cells with DHMEQ (1 mg reduced PHA-stimulated expression of t (Fig. 3a). Similarly, PHA increa and IFN-c in Jurkat cells and p cells with DHMEQ (1 mg/ml) de by approximately half (Fig. 3b)
Alessiani et al. 1991	FK 506		human			Five of eight deaths were due t Overall, 50% of patients develo 38% suffered severe ones.
Fung et al. 1991	FK 506		human			The incidence of serious infecti of FK 506, has not appeared to incidence of serious infections seen in a historical group of pa is that the incidence of cytome not appear to be increased wh patients on CyA.
Ekberg et al. 2007	cyclosporine		human			The most commonly reported s were cytomegalovirus (CMV) v infection and lymphocele (Tabl patients with opportunistic infe serious) was also similar amon cytomegalovirus infection was opportunistic infection (Table 3
Guler et al. 2011	i) IL-1RI$^{-/-}$ ii) Autologous Qb virus-like particle-based vaccines against IL-1a and IL-1b	ii) immunized s.c. three times before (at week: –5, –3 and –1) and once at week 10 post-infection	mice			
Parnet et al. 2003	IL-1RI$^{-/-}$				Activation of NFkB in response to IL-1b was no longer apparent in IL-1RI knockout mice, confirming that this receptor is essential for the transduction of IL-1 signal in the pituitary,	

Yamada et al. 2001	NF-kB p50$^{-/-}$	knockout mice	mice	
Weih et al. 1995	RelB$^{-/-}$	knockout mice	mice	RelB-deficient animals also hac immunity, as observed in conta experiments.
Lin et al. 2015	Secreted IL-1α expression		mice	Both the percent and number c CD8+ T cells, and CD69+ CD4 the expression of secreted IL-1
Nambu et al. 2006	IL-1a$^{-/-}$, IL-1b$^{-/-}$, IL-1a/b$^{-/-}$	knockout mice	mice	IL-1b, but not IL-1a, is requirec T cell activation and the inducti inflammation in the delayed-typ responses

Considerations for Potential Applications of the AOP (optional)

The impaired IL-1 signaling can lead to decreased host resistance to various infections. 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.

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Appendix 1

List of MIEs in this AOP

Event: 1700: Inhibition of IL-1 binding to IL-1 receptor (https://aopwiki.org/events/1700)

Short Name: Inhibition of IL-1 binding to IL-1 receptor

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	MolecularInitiatingEvent

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
macrophage

Organ term

Organ term
immune system

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

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).

Domain of Applicability

Taxonomic Applicability

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

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

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. 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 (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 (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.

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-1R 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)

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.

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.

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List of Key Events in the AOP

Event: 202: Inhibition, Nuclear factor kappa B (NF-kB) (https://aopwiki.org/events/202)

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 (https://aopwiki.org/aops/14)	KeyEvent
Aop:278 - IKK complex inhibition leading to liver injury (https://aopwiki.org/aops/278)	KeyEvent
Aop:277 - Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	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
macrophage

Organ term

Organ term
immune system

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

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).

Key Event Description

The NF-kB pathway consists of a series of events where the transcription factors of the NF-kB family play the key role. The NF-κB pathway can be activated by a range of stimuli, including TNF receptor activation by TNF-a, or IL-1R1 activation by IL-1a or b. Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates IkBa. This NF-kB inhibitor will be K48-linked ubiquitinated and degradated, allowing NF-kB 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-kB pathway is inhibited, its translocation will be delayed (or absent), resulting in less or no regulation of NF-kB 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). Therefore, inhibition of IL-1R1 activation suppresses activation of NF-kB.

How it is Measured or Detected

NF-kB transcriptional activity: Beta lactamase reporter gene assay (Miller et al. 2010). NF-kB transcription: Lentiviral NF-kB GFP reporter with flow cytometry (Moujalled et al. 2012)

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

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

NF-κB p65 (Total/Phospho) ELISA

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

References

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

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Event: 1702: Suppression of T cell activation (https://aopwiki.org/events/1702)

Short Name: Suppression of T cell activation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	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 (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

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

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

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.

List of Adverse Outcomes in this AOP

Event: 986: Increase, Increased susceptibility to infection (https://aopwiki.org/events/986)

Short Name: Increase, Increased susceptibility to infection

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	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

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The increased susceptibility to infection caused by IL-1RA or anti-IL-1 antibody has been reported in both humans and mice. (Fleischmann et al., 2003; De Benedetti et al., 2018; Hirsch et al., 1996)

Key Event Description

The protection of host against microbial infection depends on both innate and acquired immunity. In particular, both T cell and antibody production by B cells play a principal role.

How it is Measured or Detected

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

Regulatory Significance of the AO

After L-1R antagonist or neutralizing antibody such as IL-1Ra (generic anakinra), canakinumab (anti-IL-1b antibody) and rilonacept (soluble IL-1R) became available to treat some of autoinflammatory syndromes, it became clear that these inhibitors increased the frequency of serious bacterial infection (De Benedetti et al., 2018; 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).

References

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Chatham, W.W., 2019. Glucocorticoid effects on the immune system.

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.

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.

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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.

Migkos, M.P., Somarakis, G.A., Markatseli, T.E., et al., 2015. Tuberculous pyomyositis in a rheumatoid arthritis patient treated with anakinra. Clin Exp Rheumatol 33, 734-736.

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.

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.

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2002: Inhibition of IL-1 binding to IL-1 receptor leads to Inhibition, Nuclear factor kappa B (NF-kB) (https://aopwiki.org/relationships/2002)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

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. 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. (Brikos et al., 2007) and Weber et al. (Weber et al., 2010).

Therefore, the suppression of the binding of IL-1 to IL-1R1 suppresses activation of NF-κB.

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). Therefore, the inhibition of IL-1 binding to IL-1R1 cannot activate NF-κB.

Empirical Evidence

IL-1Ra blocks IL-1 signaling:

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

IL-1Ra suppresses 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-lra infusion synthesized significantly less interleukin 6 ex vivo than PBMC from saline-injected controls. (Granowitz et al., 1992)

Canakinumab (ACZ885, Ilaris) blocks IL-1 signaling

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) blocks IL-1 signaling:

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).

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-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)

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

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Relationship: 2003: Inhibition, Nuclear factor kappa B (NF-kB) leads to Suppression of T cell activation (https://aopwiki.org/relationships/2003)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	**adjacent**	**High**	**Moderate**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

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, PKC*u*, connects the above described TCR proximal signaling events to distal events that ultimately lead to NF-kB 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). In addition, the stimulation of T cells by IL-1 activates NF-*k*B as already described before. 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

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. (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 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).

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-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. These data suggest that IL-1b 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)
A representative NF-kB inhibitor, DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF-kB in Jurkat cells via inhibition of degradation of IkBa. Preincubation of peripheral blood mononuclear cells with DHMEQ (1 mg/ml, 3 hr) greatly reduced PHA-stimulated expression of IFN-g, IL-2 and TNF-a genes.

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 inhibits T-cell function versus infective antigenic stimuli in a dose-dependent manner in vitro (Orciuolo et al., 2007).

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Relationship: 2004: Suppression of T cell activation leads to Increase, Increased susceptibility to infection (https://aopwiki.org/relationships/2004)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of IL-1 binding to IL-1 receptor leading to increased susceptibility to infection (https://aopwiki.org/aops/277)	**adjacent**	**High**	**Not Specified**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

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.

Evidence Supporting this KER

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).

Biological Plausibility

To protect the infection from different pathogens, different types of immune response depending on the pathogens are required.

1). Type 1 immunity drives resistance to viruses and intracellular bacteria, such as *Listeria monocytogenes*, *Salmonella* spp. and *Mycobacteria* spp., as well as to intracellular protozoan parasites such as *Leishmania* spp. The T helper 1 (T_H1) signature cytokine interferon-γ (IFNγ) has a central role in triggering cytotoxic mechanisms including macrophage polarization towards an antimicrobial response associated with the production of high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), activation of $CD8^+$ cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells to kill infected cells via the perforin and/or granzyme B-dependent lytic pathway or via the ligation of surface death receptors; and B cell activation towards the production of cytolytic antibodies that target infected cells for complement and Fc receptor-mediated cellular cytotoxicity.

2) Resistance to extracellular metazoan parasites and other large parasites is mediated and/or involves type 2 immunit. Pathogen neutralization is achieved via different mechanisms controlled by T_H2 signature cytokines, including interleukin-4 (IL-4), IL-5 and IL-13, and by additional type 2 cytokines such as thymic stromal lymphopoietin (TSLP), IL-25 or IL-33, secreted by damaged cell. T_H2 signature cytokines drive B cell activation towards the production of high-affinity pathogen-specific IgG1 and IgE antibodies that function via Fc-dependent mechanisms to trigger the activation of eosinophils, mast cells and basophils, expelling pathogens across epithelia.

3) T_H17 immunity confers resistance to extracellular bacteria such as *Klebsiella pneumoniae, Escherichia coli, Citrobacter rodentium, Bordetella pertussis, Porphyromonas gingivalis* and *Streptococcus pneumoniae*, and also to fungi such as *Candida albicans, Coccidioides posadasii, Histoplasma capsulatum* and *Blastomyces dermatitidis*. Activation of T_H17 cells by cognate T cell receptor (TCR–MHC class II interactions and activation of group 3 innate lymphoid cells (ILC3s) via engagement of IL-1 receptor (IL-1R) by IL-1β secreted from damaged cells lead to the recruitment and activation of neutrophils. T_H17 immunopathology is driven to a large extent by products of neutrophil activation, such as ROS and elastase (reviewed by Soares et al. (Soares et al., 2017)).

Based on these evidences, the insufficient T cell or B cell function causes impaired resistance to infection.

Empirical Evidence

Administration of IL-1R antagonist or neutralizing antibody such as IL-1Ra (generic anakinra), canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) 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).

Since these inhibitors became available to treat some of autoinflammatory syndromes, it became clear that these inhibitors increased the frequency of serious bacterial infection (De Benedetti et al., 2018; 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).

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