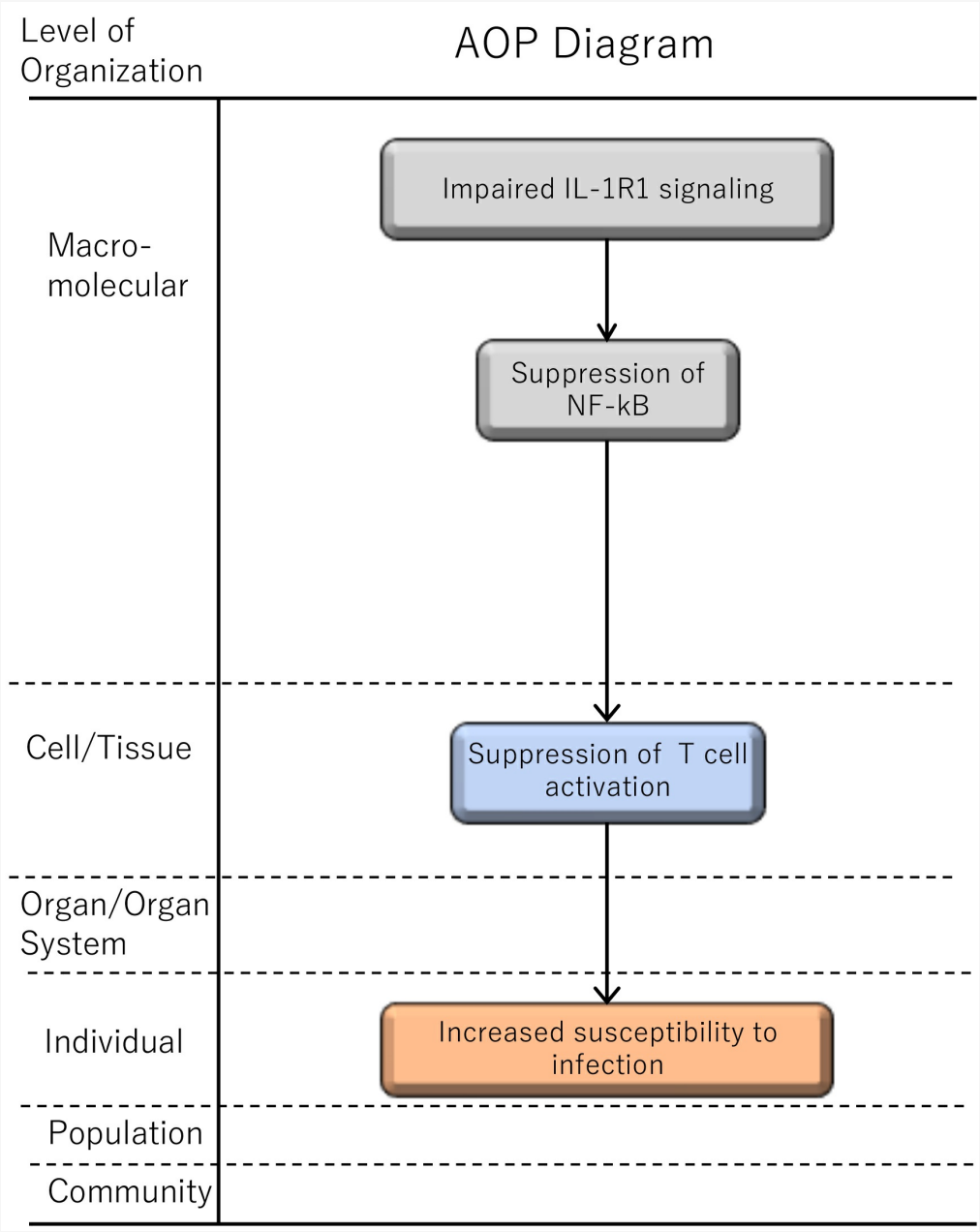
AOP ID and Title:

AOP 277: Impaired IL-1R1 signaling leading to increased susceptibility to infection
Short Title: IL-1 inhibition

Graphical Representation



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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 principal role in the immunological function of IL-1. Namely, it stimulates innate immunity such as activation of dendritic cells and macrophages. It also stimulates T cells via activated dendritic function or directly. The activation of T cells is crucial for B cell proliferation and their antibody production. The cooperation by T cells and B cells constitutes a main part of host defense against infection. Therefore, the impaired IL-1R1 signaling either by the decreased IL-1 production or the inhibition of IL-1β binding to IL-1R1 by IL-1 receptor antagonist IL-1Ra or anti-IL-1β antibody) results in the blockade of the effects of the pleiotropic cytokine IL-1β leading to increased susceptibility to infection.

In this AOP, we selected the impaired IL-1R signaling as a molecular initiating event (MIE), and suppression of NF-κB, suppression of T cell activation, and increased susceptibility to infection as key events (KE).

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).

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

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

Summary of the AOP

Events

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

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

Sequence	Type	Event ID	Title	Short name
4	AO	936	Increase, Increased susceptibility to infection	Increase, Increased susceptibility to infection

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Impaired IL-1R1 signaling	adjacent	Inhibition, Nuclear factor kappa B (NF-kB)	High	Moderate
Inhibition, Nuclear factor kappa B (NF-kB)	adjacent	Suppression of T cell activation	High	Moderate
Suppression of T cell activation	adjacent	Increase, Increased susceptibility to infection	High	High

Stressors

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

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	High

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence

Mixed High
Sex Evidence

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

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

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

275 organisms have orthologs with human gene NFKB1.

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

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, Jin and Dubin, 2017; Yamada et al., 2000).

IL-1 receptor antagonist IL-1Ra was purified in 1990, and the cDNA reported that same year. IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (generic anakinra) is fully active in blocking the IL-1R1, and therefore, the activities of IL-1α and IL-1β. Anakinra is approved for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndrome (CAPS). Since its introduction in 2002 for the treatment of rheumatoid arthritis, anakinra has had a remarkable record of safety. However, Fleischmann et al. (Fleischmann et al., 2003) reported that serious infectious episodes were observed more frequently in the anakinra group (2.1% versus 0.4% in the placebo group) and other authors reported the increased susceptibility to bacterial or tuberculosis infection (Genovese et al., 2004; Kullenberg et al., 2016; Lequerre et al., 2008; 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 (von Bernuth et al., 2012).

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

Weight of Evidence Summary

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

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

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

Biological plausibility

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

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

The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1RI that facilitates recruitment of IL-1R3. the trimeric complex rapidly assembles two intracellular signaling proteins, myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor–activated protein kinase (IRAK) 4. This is paralleled by the (auto)phosphorylation of IRAK4, which subsequently phosphorylates IRAK1 and IRAK2, and then this is followed by the recruitment and oligomerization of tumor necrosis factor–associated factor (TRAF) 6. Activation of NF-κB by IL-1 requires the activation of inhibitor of nuclear factor B (IκB) kinase 2 (IKK2). Activated IKK phosphorylates IκBα, which promotes its K48-linked polyubiquitination and subsequent degradation by the proteasome. IκB destruction allows the release of p50 and p65 NF-κB subunits and their nuclear translocation, which is the central step in activation of NF-κB. Both NF-κBs bind to a conserved DNA motif that is found in numerous IL-1–responsive genes. (Weber et al., 2010a, b)

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

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

Suppression of T cell activation leads to 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

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

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

1-1. **Decreased IL-1 production**

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

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

Other than the inhibitors for NF-κB signaling, which can be stimulated by various stimulations other than TLR4 stimulation, there are signaling molecules that are specific to TLR4 signaling, such as TLR4, Mal, TRAM, Myd88, IRAK4, and IRAK1/2 (Vallabhapurapu

and Karin, 2009). There are several chemicals that targe some of these molecules, an inhibitors of TLR4 such as TAK-242 (Matsunaga et al., 2011) and various IRAK4 inhibitors (Lee et al., 2017). IRAK4 has recently attracted attention as a therapeutic target for inflammation and tumor diseases (Chaudhary, Robinson and Romero, 2015).

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

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

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

Several reports described that the administration of IL-1 receptor antagonist IL-1Ra , canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) led to increased susceptibility to infection (De Benedetti et al., 2018; Fleischmann et al., 2003; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Migkos et al., 2015; Schlesinger et al., 2012; Yokota et al., 2017).

1-3. **IL-1 deficient mice**

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, Jin and Dubin, 2017; Yamada et al., 2000). Moreover, polymorphism of IL-1β or IL-1Ra leads to the increased susceptibility to tuberculosis, severe sepsis or fungal infection (Fang et al., 1999; Motsinger-Reif et al., 2010; Wojtowicz et al., 2015). RelB deficient mice had an impaired cellular immunity, as observed in contact sensitivity reaction (Weih et al., 1995).

Quantitative Consideration

IL-1Ra blocks IL-1 signaling:

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

Canakinumab (ACZ885, Ilaris):

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

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

Rilonacept (IL-1 Trap, Arcalyst):

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

Considerations for Potential Applications of the AOP (optional)

The impaired IL-1 signaling can lead to 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: Impaired IL-1R1 signaling

Short Name: Impaired IL-1R1 signaling

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Impaired IL-1R1 signaling leading to increased susceptibility to infection	MolecularInitiatingEvent

Stressors

Name
IL-1 receptor antagonist IL-1Ra (Anakinra)
anti-IL-1b antibody (Canakinumab)
soluble IL-1R (Rilonacept)
curcumin
iguratimod
epigallocathechin gallate
TAK-242
IRAK4 inhibitors
Dehydroxymethylepoxyquinomicin (DHMEQ)
Dimethyl fumarate
anti-IL-1b antibody (Gevokizumab)

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
macrophage

Organ term

Organ term
immune system

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

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

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

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

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

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

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

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

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

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

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

In LPS-induced acute lung injury (ALI) mice model, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were significantly decreased by cinnamaldehyde (CA) (Huang and Wang, 2017).

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

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

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

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

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence

Unspecific **Sex** High **Evidence**

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

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

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

1. **Decreased IL-1 production**

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

Binding of LPS to TLR4 and the coreceptor MD2 triggers interactions between the cytoplasmic TIR domain of TLR4 and TIR-containing adaptor proteins (Mal, MyD88, and TRAM). MyD88 binds IRAK4, which requires its kinase activity to bind the kinases IRAK1 and IRAK2 sequentially. The MyD88–IRAK complex also engages the ubiquitin ligase TRAF6 to make polyubiquitin chains that activate the IKK complex for NF-kB- and ERK-dependent gene transcription. Ubiquitin ligases cIAP1 and cIAP2 recruited to the TLR4 signaling complex regulate translocation of a subset of signaling components to the cytoplasm, where TAK1 activation initiates a MAPK cascade, p38a and JNK, which stimulates gene expression. TLR4 activated at the plasma membrane is endocytosed but can signal within the endosomal compartment via the adaptors TRAM and TRIF. The kinase and ubiquitin ligase combination of RIP1 and Peli1 interacts with TRIF to signal NF-kB activation, whereas TBK1 and TRAF3 stimulate IRF3-dependent transcription. Through these signaling cascades, NF-κB, activator protein-1 (AP-1), cAMP responsive element binding protein (CREB)/ activating transcription factor (ATF), CCAAT-enhancer-binding protein b (c/EBP b), and interferon regulatory factor 3 (IRF3) are activated. These transcription factors induce the expression of various inflammatory cytokines e.g., IL-1β, TNFα, IL-6 and several chemokines (reviewed by Newton and Dixit (Newton and Dixit, 2012)).

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

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

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

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

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

How it is Measured or Detected

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

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List of Key Events in the AOP

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

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

Key Event Component

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

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:14 - Glucocorticoid Receptor Activation Leading to Increased Disease Susceptibility	KeyEvent
Aop:278 - IKK complex inhibition leading to liver injury	KeyEvent
Aop:277 - Impaired IL-1R1 signaling leading to increased susceptibility to infection	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
Mus musculus	Mus musculus	High	NCBI
Rattus norvegicus	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

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

Evidence for perturbation of this molecular initiating event by stressor

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

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

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

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

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

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

In LPS-induced acute lung injury (ALI) mice model, LPS induced inflammatory cytokines such as TNF-α, IL-6, IL-13 and IL-1β were significantly decreased by cinnamaldehyde (CA) (Huang and Wang, 2017).

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

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

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

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

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

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

Key Event Description

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

How it is Measured or Detected

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

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

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

NF-κB p65 (Total/Phospho) ELISA

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

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Event: 1702: Suppression of T cell activation

Short Name: Suppression of T cell activation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Impaired IL-1R1 signaling leading to increased susceptibility to infection	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

T cell

Organ term

Organ term

immune system

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Description

T cells are key orchestrators of the response against pathogens and are also fundamental in maintaining self-tolerance. A number of clinically important conditions have been described in which T-cell functions are altered, as in AIDS or upon immunosuppression after application of various immunosuppressive drugs to treat autoimmune disorders or allogeneic graft rejection. T-cell progenitors differentiate in the thymus into immature T cells that acquire the expression of the T-cell receptor (TCR), which recognizes antigen peptides from pathogens presented along with major histocompatibility complex (MHC). In addition to the TCR, T cells are characterized by expression of the co-receptor molecules CD4 and CD8 on their cell surface. CD4+ T cells, also called T helper (Th) cells, recognize antigen/MHC-II complexes on antigen presenting cells (APCs) and coordinate the activation of other immune cells including B cells, macrophages, etc.

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

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

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

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

How it is Measured or Detected

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

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List of Adverse Outcomes in this AOP

Event: 986: Increase, Increased susceptibility to infection

Short Name: Increase, Increased susceptibility to infection

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:277 - Impaired IL-1R1 signaling leading to increased susceptibility to infection	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
Mus musculus	Mus musculus	High	NCBI
Rattus norvegicus	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

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

Key Event Description

Severe combined immunodeficiencies (SCIDs) comprise a group of rare, monogenic diseases that are characterized by an early onset and a profound block in the development of T lymphocytes. Given that adaptive immunity is abrogated, patients with SCID are prone to recurrent infections caused by both non-opportunistic and opportunistic pathogens, leading to early death unless immunity can be restored reviewed by Fischer et al. (Fischer et al., 2015) . Human immunodeficiency virus (HIV) is a retrovirus known to attack the CD4+ T lymphocytes. In individuals with chronic HIV infection not on treatment with antiretroviral agents, as the CD4+ count drops they are vulnerable to a multitude of infections which rarely occur in an immunocompetent host, hence the term opportunistic infections (Justiz Vaillant and Naik, 2021). Various immunosuppressive agents such as corticosteroids, antimetabolites, calcineurin inhibitors, glucocorticoids, antithymocyte globulin, antibodies against IL-2RA or CD28, which all suppress T cell function, increase the incidence of opportunistic infection (reviewed by Tasdogan et al. (Tasdogan et al., 2019).

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

Increased susceptibility to infection is the significant adverse outcome of drugs and chemicals present in the environment. Therefore, drugs or chemicals that have an effect that can cause immunosuppression must be under regulatory scrutiny.

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Impaired IL-1R1 signaling leading to increased susceptibility to infection	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

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

Evidence Supporting this KER

Mice lacking MYD88 or IRAK4 show severe defects in IL-1 signaling (Adachi et al., 1998; 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).

Biological Plausibility

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

Empirical Evidence

IL-1Ra blocks IL-1 signaling:

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

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

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

IL-1Ra competed for binding of ^{125}I-IL-1a to type I IL-1R present on EL4 thymoma cells, 3T3 fibroblasts, hepatocytes, and Chinese hamster ovary cells expressing recombinant mouse type I IL-1R. The IC50 values for IL-1ra binding (ranging from 2 to 4

ng/ml) were similar to those of IL-1a. (McIntyre et al., 1991)

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

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

Canakinumab (ACZ885, Ilaris):

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

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

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

Rilonacept (IL-1 Trap, Arcalyst):

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

IRAK4 inhibitor

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

Various concentrations of kinase-active or kinase-inactive IRAK-4 were transiently (Lye et aloverexpressed in IRAK-4-deficient cells that were also transiently transfected with an NF-κB-dependent luciferase reporter and α-galactosidase expression vector. Transfected cells were left untreated or treated with IL-1β (10 ng/ml) for 6 h before luciferase and α-galactosidase activities were measured. The luciferase activity was divided by the α-galactosidase activity, and fold activation was calculated compared with the activity of untreated cells carrying an empty α-vector (normalized as 1). The results demonstrated that kinase-active IRAK-4 dose dependently activates NF-κB (Lye et al., 2004).

Quantitative Understanding of the Linkage

See Empirical Evidence.

Response-response relationship

IL-1Ra blocks IL-1 signaling:

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

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

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

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Impaired IL-1R1 signaling leading to increased susceptibility to infection	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

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

In T cells, NF-kB can be activated by several pathways of signal transduction. The engagement of the TCR by major histocompatibility complex (MHC) plus antigen initiates downstream CD3 immunotyrosine activation motif (ITAM) phosphorylation by the Src family kinases, FYN and leukocyte C-terminal src kinase (LCK). Phosphorylated CD3 activates the T cell specific tyrosine kinase, zeta-chain associated protein kinase (ZAP-70), which ultimately trigger calcium release and protein kinase (PK)C activation, respectively. Activation of a specific PKC isoform, PKCμ, connects the above described TCR proximal signaling events to distal events that ultimately lead to NF-kB activation. Importantly, PKCm activation is also driven by engagement of the T cell co-stimulatory receptor CD28 by B7 ligands on antigen presenting cells (APCs). In addition, the stimulation of T cells by IL-1 activates NF-kB as already described before. Once in the nucleus, NF-kB governs the transcription of numerous genes involved in T cell survival, proliferation, and effector functions (Paul and Schaefer, 2013).

Evidence Supporting this KER

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

Biological Plausibility

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

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

Delayed-type hypersensitivity (DTH) responses were significantly suppressed in IL-1b-deficient and IL-1a/b-deficient mice. Lymph node cells derived from antigen-sensitized IL-1b-deficient and IL-1a/b-deficient mice and IL-1R type I-deficient mice, exhibited reduced proliferative responses against antigen. (Nambu et al., 2006).

Empirical Evidence

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

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

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

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

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

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

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

Quantitative Understanding of the Linkage

A representative NF-kB inhibitor, MG132 that suppresses NF-kB activity at more than 10 mM (Fiedler et al. 1998) suppresses IL-2-

induced activation of STAT5 at 50 mM. (Yu and Malek., 2001).

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 (Nishioka et al., 2008).

Response-response relationship

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

Bortezomib 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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Impaired IL-1R1 signaling leading to increased susceptibility to infection	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

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

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 (reviewed by Soares et al. (Soares et al., 2017)).

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.

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).

Certain pharmaceutical agents known as calcineurin inhibitors that suppress T cell function and are commonly used to prevent organ rejection of transplant recipients or to treat autoimmune disorders, also have an immunosuppressive side effect, known to

lead to an increase in the following opportunistic infections: fungal/yeast (e.g., Cryptococcus neoformans); viral (esp. herpes-family viruses such as Epstein-Barr virus [EBV], cytomegalovirus [CMV]); atypical mycobacterial (e.g., mycoplasma, Nocardia, Listeria, mycobacteria); and parasitic (e.g., toxoplasmosis) infections (reviewed by Singh (2005)).

Quantitative Understanding of the Linkage

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

Response-response relationship

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

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