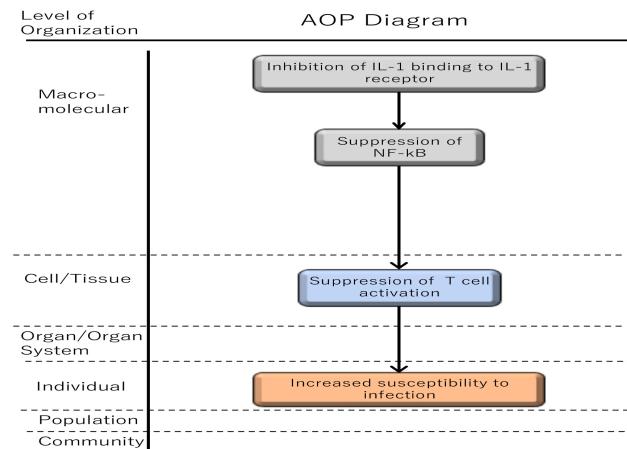


AOP ID and Title:

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

Short Title: IL-1 inhibition

Graphical Representation



Authors

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Abstract

The pleiotropic cytokine IL-1 mediates its biological functions via association with the signaling receptor IL-1R1. These may include initiation of innate immunity as well as acquired immunity, which are essential for assistance of host defense against infection. The trimeric complex consists of IL-1, IL-1R1 and IL-1R3 (a coreceptor, formerly IL-1R accessory protein) allows for the approximation of the Toll-IL-1-Receptor (TIR) domains of each receptor chain. MyD88 then binds to the TIR domains. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB. The activation of NF-κB plays a principle role in the immunological function of IL-1. Namely, it stimulates innate immunity such as activation of dendritic cells and macrophages. It also stimulates T cells via activated dendritic function or directly. The activation of T cells is crucial for B cell proliferation and their antibody production. The cooperation by T cells and B cells constitutes a main part of host defense against infection.

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

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- κ B) (https://aopwiki.org/events/202)	Inhibition, Nuclear factor kappa B (NF- κ B)
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- κ B)	High	Moderate
Inhibition, Nuclear factor kappa B (NF- κ B) (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>), 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> (<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-1 α and IL-1 β . 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; Migkots et al., 2015). Two IL-1 signaling antagonists, canakinumab (anti-IL-1 β 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- κ B p50 are unable effectively to clear *L. monocytogenes* and are more susceptible to infection with *S. pneumoniae* (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-1 α or IL-1 β to IL-1R to the activation of NF- κ B through the assemble of MyD88 to the trimelic complex composed of IL-1, IL-1R1, 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 PGE₂ (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- κ B)

IL-1 α and IL-1 β 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-1R1 complexes.

The initial step in IL-1 signal transduction is a ligand-induced conformational change in the first extracellular domain of the IL-1R1 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- κ B by IL-1 requires the activation of inhibitor of nuclear factor B (I κ B) kinase 2 (IKK2). Activated IKK phosphorylates I κ B α , which promotes its K48-linked polyubiquitination and subsequent degradation by the proteasome. I κ B destruction allows the release of p50 and p65 NF- κ B subunits and their nuclear translocation, which is the central step in activation of NF- κ B. Both NF- κ Bs 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- κ B) leads to Suppression of T cell activation

In T lineage cells, the temporal regulation of NF- κ B 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-1 β . (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 cells. 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 (ILCs3) via engagement of IL-1 receptor (IL-1R) by IL-1 β 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.

Reference	Chemical Initiator or deleted gene	dose	Species	MIE	KE1	KE2
Dripps et al. 1991	IL-1Ra (anakinra)			Inhibition of IL-1 binding to IL-1 receptor Equilibrium binding and kinetic experiments show that IL-1 α binds to the 80-kDa IL-1 receptor on the murine thymomae II line EL4 with an affinity (K_D = 150 pM) approximately equal to that of IL-1 α and IL-1 β for this receptor Determined by its ability to inhibit the IL-1 α stimulation of murine D10S cell. The expected ED ₅₀ is 20-40 ng/ml in the presence of 50 pg/ml of IL-1 α .	Inhibition, Nuclear factor kappa B (NF- κ B)	Suppression of T cell activation
Sigma-Aldrich Specification Sheet	IL-1Ra (anakinra)					
Fleischmann et al. 2003	IL-1Ra (anakinra)	100 mg of anakinra or placebo, administered daily by subcutaneous injection	human	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		
Genovese et al. 2004	IL-1Ra (anakinra)					
Kullenberg et al. 2016	IL-1Ra (anakinra)		human	administered as daily s.c. injections		
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- κ B 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- κ B 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- κ B activation.	
Dhimolea et al. 2010	canakinumab				Canakinumab binds to human IL-1 β with high affinity; the antibody-antigen dissociation equilibrium constant is approximately 35-40 pM.	
De Benedetti et al. 2018	canakinumab	150 mg subcutaneously every 4 weeks	human		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.	
Imagawa et al. 2013	canakinumab	either 150 mg s.c. or 2 mg/kg for patients with a body weight \leq 40 kg	human			
Lachmann et al. 2009	canakinumab	every 8 weeks for 24 weeks received 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	rilonacept (Sixth Edition), 2011		human	Rilonacept has a very high binding affinity for IL-1 (dissociation constant ~1 pM), and it is specific for IL-1 β and IL-1 α .
Hoffman et al. 2008	rilonacept	weekly subcutaneous injections (160 mg)	human	
Roell et al. 2010	gevokizumab (XOMA 052)		human	XOMA 052 neutralizes IL-1 β stimulation of NF κ B activation in HeLa cells stably expressing an NF κ B-luciferase reporter construct with an IC ₅₀ of ~1 pM at the EC ₅₀ for this assay (25 pg/ml IL-1 β).
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- κ B (NF- κ B) luciferase reporter plasmid)	an average K _B value (mean \pm S.D., n=3) of 4.8 \pm 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). ALLN (Fig. 3A) and MG132 (Fig. 3B) (10 mg/mL = 21mM) reduced the cytokine-mediated NF κ B activation.
Hellerbrand et al. 1998	MG-132		rat (in vitro)	In all cell lines, giotoxin, MG132 (10 mM) or sulfasalazine strongly reduced VP16-induced NF- κ B-driven luciferase expression.
Arlt et al. 2001	MG-132		human (in vitro)	The increase in NF- κ B 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- κ B induced by LPS+PMA was blocked by MG132.
Ortiz-Lazareno et al. 2008	MG-132		human (in vitro)	
Yu and Malek 2001	MG-132		mice (in vitro)	MG132 (50mM) stabilized IL-2 phosphorylated STAT5, which after 2 h in culture (Fig. 5A, lane CMV-specific cytotoxicity of CD decreased in the presence of λ . In vivo MG132 administration to DNFB-induced dermatitis reduced maintained the level of Th1 cell alleviation of dermatitis lesions serum IgE hyperproduction apparently inhibits the growth of a cells both in vivo and in vitro the percentage of CD69/TNF α with the increment of bortezom
Wang et al. 2011	MG-132		human (in vitro)	
Ohkusu-Tsukada et al. 2018	MG-132	repeatedly i.p. injected 200 nmol of MG132 on days 0, 3, 5, 7, 9, mice (in vivo) 11, 13, 15, 17, and 19.		
Satou et al. 2004	bortezomib		human (in vitro, in vivo)	
Orciolo et al. 2007	bortezomib	0.1 mM, 1 mM, 10 mM	human (in vitro)	
Matsumoto et al. 2005	dehydroxymethyl epoxyquinomicin (DHMEQ)		human	The addition of DHMEQ (10 mg/mL) completely inhibited the activated NF- κ B for at least 8 hours.
Nishioka et al. 2008	dehydroxymethyl epoxyquinomicin (DHMEQ)		human (in vitro)	DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF- κ B in Jurkat cells via inhibition of degradation of I κ B α . Exposure of PBMC to PHA greater expression of IFN- γ , IL-2 and TNF- α (Fig. these cells with DHMEQ (1 mg/mL) reduced PHA-stimulated expression of t (Fig. 3a). Similarly, PHA increased and IFN- γ in Jurkat cells and p cells with DHMEQ (1 mg/mL) decreased by approximately half (Fig. 3b). Five of eight deaths were due to sepsis. Overall, 50% of patients developed 38% suffered severe ones. The incidence of serious infections of FK 506, has not appeared to be increased compared to patients on CyA. The most commonly reported serious infections were cytomegalovirus (CMV) infection and lymphocytopenia (Table 3). Patients with opportunistic infections (serious) was also similar among patients with CMV infection (Table 3).
Alessiani et al. 1991	FK 506		human	
Fung et al. 1991	FK 506		human	
Ekberg et al. 2007	cyclosporine		human	
Guler et al. 2011	i) IL-1RI $^{-/-}$ ii) Autologous Qb virus-like particle-based vaccines against IL-1 α and IL-1 β	ii) immunized s.c. three times before (at week: -5, -3 and -1) and once at week 10 post-infection		Activation of NF κ B in response to IL-1 β 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.
Parnet et al. 2003	IL-1RI $^{-/-}$			

Yamada et al. 2001	NF- κ B p50 $^{−/−}$	knockout mice	mice	
Weih et al. 1995	RelB $^{−/−}$	knockout mice	mice	RelB-deficient animals also have immunity, as observed in control experiments.
Lin et al. 2015	Secreted IL-1 α expression		mice	Both the percent and number of CD8+ T cells, and CD69+ CD4+ cells express the expression of secreted IL-1 IL-1b, but not IL-1a, is required for T cell activation and the induction of inflammation in the delayed-type 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)

AOP277

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

How it is Measured or Detected

1. Competitive inhibition binding experiments using ^{125}I -IL-1 α 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-1 β 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- κ B) (<https://aopwiki.org/events/202>)

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

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- κ B signaling, resulting in differential production of cytokines and chemokines (McKay and Cidlowski, 1999; Pernis, 2007). 17 β -estradiol regulates pro-inflammatory responses that are transcriptionally mediated by NF- κ B through a negative feedback and/or transpressive interaction with NF- κ B (Straub, 2007). Progesterone suppresses innate immune responses and NF- κ B signal transduction reviewed by Klein et al. (Klein and Flanagan, 2016). Androgen-receptor signaling antagonises transcriptional factors NF- κ B(McKay and Cidlowski, 1999).

Key Event Description

The NF- κ B pathway consists of a series of events where the transcription factors of the NF- κ B family play the key role. The NF- κ B pathway can be activated by a range of stimuli, including TNF receptor activation by TNF- α , or IL-1R activation by IL-1 α or β . Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates I κ B α . This NF- κ B inhibitor will be K48-linked ubiquitinated and degraded, allowing NF- κ B 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 I κ B α and A20. When the NF- κ B pathway is inhibited, its translocation will be delayed (or absent), resulting in less or no regulation of NF- κ B 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- κ B.

How it is Measured or Detected

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

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

I κ B α phosphorylation: Western blotting (Miller et al. 2010)

NF- κ B p65 (Total/Phospho) ELISA

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

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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)

AOP277

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 1 regulatory 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 [³H]thymidine incorporation.

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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 IL-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).

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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- κ B) (<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-1R1 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-1R1, IL-1RacP, 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- κ B. 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- κ B.

Evidence Supporting this KER

Biological Plausibility

IL-1 α and IL-1 β 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-1 α and IL-1 β . The binding of IL-1 α and IL-1 β to IL-1R1 can be suppressed by soluble IL-R like rilonacept. The binding of IL-1 β to IL-1R1 can also be inhibited by anti-IL-1 β antibody (anti-IL-1 β antibody). Therefore, the inhibition of IL-1 binding to IL-1R1 cannot activate NF- κ B.

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 rIL-1 α -induced mouse thymocytes proliferation (ED50 almost 3 mg/mL) (Arend et al., 1990)

IL-1Ra competed for binding of 125 IL-1 α 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-1 α binding (ranging from 2 to 4 ng/ml) were similar to those of IL-1 α . (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 ($0.7 \times 10(6)$ - $1.1 \times 10(6)$ units/mg). (Shuck et al., 1991)

Peripheral blood mononuclear cells (PBMC) obtained after completion of the IL-1 α 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-1 β with high affinity; the antibody-antigen dissociation equilibrium constant is approximately 35–40 pM (Dhimolea, 2010).

The antibody binds to human IL-1 β with high affinity (about 40 pmol/l). The antibody was found to neutralize the bioactivity of human IL-1 β 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-1 β producing cells completely suppressed joint swelling (0.06 mg/kg of EC50) (Alten et al., 2008).

Primary human fibroblasts are stimulated with recombinant IL-1 β 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) blocks IL-1 signaling

Incubation of the human MRC5 fibroblastic cell line with IL-1 β induces secretion of IL-6. At a constant amount of IL-1 β (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-1 β , but also blocks IL-1 α with high affinity (KD = ~3 pM; data not shown). The titration curve of IL-1 trap in the presence of 10 pM IL-1 β 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-1Ra inhibited IL-1-induced IL-1 or TNFa synthesis by 50% (P < .01); an equimolar concentration of IL-1Ra inhibited synthesis of these two cytokines by over 20% (P < .05). A 10-fold molar excess of IL-1Ra over IL-1b reduced IL-1b-induced IL-1a by 95% (P = .01) and IL-1a-induced IL-1b by 73% (P < .01). In elutriated monocytes, a 10-fold molar excess of IL-1Ra reduced IL-1b-induced IL-1a 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-1 β 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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Lachmann, H.J., Kone-Paut, I., Kuemmerle-Deschner, J.B., et al., 2009. Use of canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med* 360, 2416-2425.

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.

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Relationship: 2003: Inhibition, Nuclear factor kappa B (NF- κ B) 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- κ B 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 (PKC) activation, respectively. Activation of a specific PKC isoform, PKC ζ , connects the above described TCR proximal signaling events to distal events that ultimately lead to NF- κ B activation. Importantly, PKC ζ 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- κ B as already described before. Once in the nucleus, NF- κ 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

AOP277

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- κ B inhibitors have been reported. MG132, bortezomib, curcumin, DHMEQ(Dehydroxymethylepoxyquinomicin), naringin, sorafenib, genistein and parthenolide are some of representatives (Pordanjani and Hosseiniemehr, 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 μ M. 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 (Orciulo et al., 2007).

DHMEQ, a novel nuclear factor- κ B 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- κ B 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- κ B inhibitor, MG132 that suppresses NF- κ B 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- κ B inhibitor, DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF- κ B in Jurkat cells via inhibition of degradation of I κ B α . Preincubation of peripheral blood mononuclear cells with DHMEQ (1 mg/ml, 3 hr) greatly reduced PHA-stimulated expression of IFN- γ , IL-2 and TNF- α 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 μ M. 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 (Orciulo 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 (Th1) signature cytokine interferon- γ (IFN 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 (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 immunity. Pathogen neutralization is achieved via different mechanisms controlled by Th2 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. Th2 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 (ILCs3s) via engagement of IL-1 receptor (IL-1R) by IL-1 β 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., 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-1 β 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 PGE₂ (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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