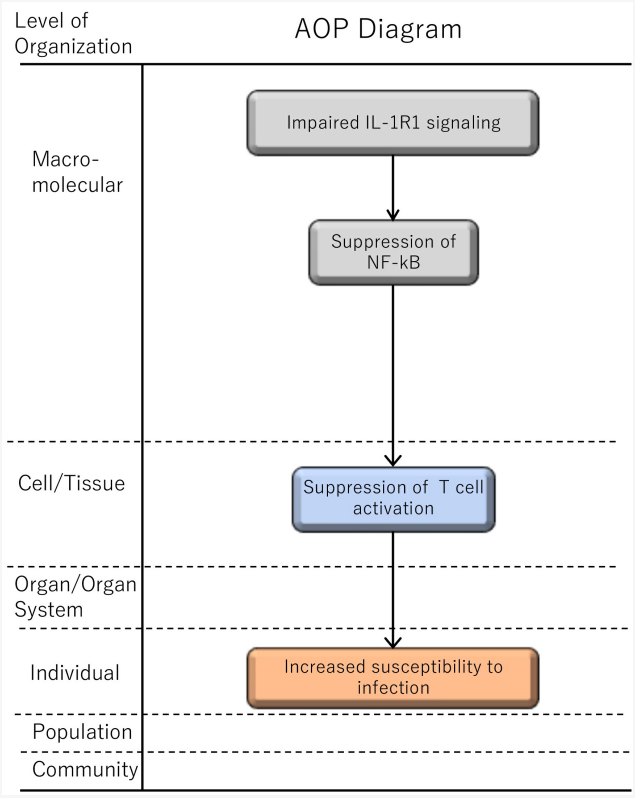
AOP ID and Title:

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

Graphical Representation



Authors

Yutaka Kimura (1) Setsuya Aiba (1)

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

Corresponding author: Setsuya Aiba

Status

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

Abstract

The pleiotropic cytokine IL-1 mediates its biological functions via association with the signaling receptor IL-1R1. These may include initiation of innate immunity as well as acquired immunity, which are essential for assistance of host defense against infection. The trimeric complex consists of IL-1, IL-1R1 and IL-1R3 (a coreceptor, formerly IL-1R accessory protein) allows for the approximation of the Toll-IL-1-Receptor (TIR) domains of each receptor chain. MyD88 then binds to the TIR domains. The binding of MyD88 triggers a cascade of kinases that produce a strong pro-inflammatory signal leading to activation of NF-κB. The activation of NF-κB plays a 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-1b 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-1b 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-1b production under different pathophysiological conditions (Bent et al., 2018).

Therefore, the inhibition of various targets in different layers from the stimulation of PRPs 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-1b 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).

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
4	AO	986	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	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
Dexamethasone	High
minocycline	High
Belnacasan (VX-765)	High
Pralnacasan (VX-740, HMR3480)	High
cinnamic aldehyde	High

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	High

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Mixed	High

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

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

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

275 organisms have orthologs with human gene NFKB1.

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

The 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)

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

Essentiality of the Key Events

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

IL-1 receptor antagonist IL-1Ra was purified in 1990, and the cDNA reported that same year. IL-1Ra binds IL-1R but does not initiate IL-1 signal transduction (Dripps et al., 1991). Recombinant IL-1Ra (generic anakinra) is fully active in blocking the IL-1R1, and therefore, the activities of IL-1α and IL-1β. Anakinra is approved for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndrome (CAPS). Since its introduction in 2002 for the treatment of rheumatoid arthritis, anakinra has had a remarkable record of safety. However, Fleischmann et al. (Fleischmann et al., 2003) reported that serious infectious episodes were observed more frequently in the anakinra group (2.1% versus 0.4% in the placebo group) and other authors reported the increased susceptibility to bacterial or tuberculosis infection (Genovese et al., 2004; Kullenberg et al., 2016; Lequerre et al., 2008; Migkos et al., 2015). Two IL-1 signaling antagonists, canakinumab (anti-IL-1b antibody) and rilonacept (soluble IL-1R) had been reported to increase susceptibility to infection (De Benedetti et al., 2018; Imagawa et al., 2013; Lachmann et al., 2009; Schlesinger et al., 2012).

In a similar way, defect of MyD88 signaling caused by knockout of mice gene or deficiency in human patient leads to the increased susceptibility to bacterial or tuberculosis infection (Fremond et al., 2004; Picard et

al., 2010; Scanga et al., 2004; von Bernuth et al., 2008). Although MyD88 is also known to be involved in TLR signaling pathway, several reports suggested that MyD88-dependent response was IL-1 receptor-mediated but not TLR-mediated. These data suggest to essentiality of IL-1-MyD88 signaling pathway in host defense against infection.

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

Weight of Evidence Summary

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

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

Biological plausibility

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

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

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

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

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

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

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

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

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

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

Empirical support

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

concordance table empirical data						
				MIE	KE1	KE2
Reference	Chmical Initiator or deleted gene	dose	Species	Inhibition of IL-1 binding to IL-1 receptor	Inhibition, Nuclear factor kappa B (NF-kB)	Suppression of T cell activatio
Dripps et al. 1991	IL-1Ra (anakinra)			Equilibrium binding and kinetic experiments show that IL-1ra binds to the 80-kDa IL-1 receptor on the murine thymomcae ll line EL4 with an affinity (K_D = 150 pM) approximately equal to that of IL-la and IL-1b for this receptor		
Sigma-Aldrich Specification Sheet	IL-1Ra (anakinra)			Determined by its ability to inhibit the IL-1alpha stimulation of murine D10S cell. The expected ED50 is 20-40 ng/ml in the presence of 50 pg/ml of IL-1alpha.		
Fleischmann et al. 2003	IL-1Ra (anakinra)	100 mg of anakinra or placebo, administered daily by subcutaneous injection	human			
Genovese et al. 2004	IL-1Ra (anakinra)	treated with subcutaneous etanercept only (25 mg twice weekly), full-dosage etanercept (25 mg twice weekly) plus anakinra (100 mg/day), or half-dosage etanercept (25 mg once weekly) plus anakinra (100 mg/day) for 6 months	human			
Kullenberg et al. 2016	IL-1Ra (anakinra)	administered as daily s.c. injections	human			
Lequerre et al. 2008	IL-1Ra (anakinra)	treated with anakinra (1–2 mg/kg/day in children, 100 mg/day in adults)	human			
Migkos et al. 2015	IL-1Ra (anakinra)		human			
Settas et al. 2007	IL-1Ra (anakinra)		human			
Lee et al. 2004	IL-1Ra (anakinra)		intrathecal administration of IL-1ra (6 mg)		intrathecal pretreatment with IL-1ra (6 mg) or YVAD (0.5 mg) significantly inhibited NF-kB DNA-binding activity upregulation bilaterally (Fig. 3C). The intrathecal administration of IL-1ra or YVAD into non-inflamed animals produced no significant change in the DNA-binding activity of NF-kB p65.	
Vallejo et al. 2014	IL-1Ra (anakinra)	In diabetic rats treated with anakinra (100 or 160 mg/Kg/day for 3 or 7 days before sacrifice)	rat		In diabetic rats treated with anakinra (100 or 160 mg/Kg/day for 3 or 7 days before sacrifice) a partial improvement of diabetic endothelial dysfunction occurred, together with a reduction	

					of vascular NADPH oxidase and NF-κB activation.	
Dhimolea et al. 2010	canakinumab			Canakinumab binds to human IL-1β with high affinity; the antibody-antigen dissociation equilibrium constant is approximately 35–40 pM. Cmax was 1.2, 1.2 and 1.5 pM for 1, 3 and 10 mg/kg antibody respectively, at days 42–56 after the first infusion.		
De Benedetti et al. 2018	canakinumab	150 mg subcutaneously every 4 weeks	human			
Imagawa et al. 2013	canakinumab	either 150 mg s.c. or 2 mg/kg for patients with a body weight ≤ 40 kg every 8 weeks for 24 weeks received	human			
Lachmann et al. 2009	canakinumab	150 mg of canakinumab subcutaneously every 8 weeks for up to 24 weeks	human			
Schlesinger et al. 2012	canakinumab	one dose of canakinumab 150 mg	human			
Textbook of Pediatric Rheumatology (Sixth Edition), 2011	rilonacept		human	Rilonacept has a very high binding affinity for IL-1 (dissociation constant ~1 pM), and it is specific for IL-1β and IL-1α.		
Hoffman et al. 2008	rilonacept	weekly subcutaneous injections (160 mg)	human			
Roell et al. 2010	gevokizumab (XOMA 052)		human		XOMA 052 neutralizes IL-1b stimulation of NFkB activation in HeLa cells stably expressing an NFkB-luciferase reporter construct with an IC_{50} of ~1 pM at the EC_{50} for this assay (25 pg/ml IL-1b).	
Mansouri et al. 2015	gevokizumab (XOMA 052)	receive gevokizumab 60 mg subcutaneously every 4 weeks for a total of three injections (12 weeks) with a 4-week follow-up period	human			
Issafras et al. 2014	gevokizumab (XOMA 052)		human (HeLa cells stably transfected with a nuclear factor-kB (NF-kB) luciferase reporter plasmid)		an average K_B value (mean±S.D., n=3) of 4.8±4.4 pM	
Palombella et al. 1994	MG-132		human (in vitro)		Both MG115 and MG132 (at 20-40 mM) markedly inhibited the formation of p50 in HeLa S100 extracts (Figure 4A, lanes 8-13).	
Hellerbrand et al. 1998	MG-132		rat (in vitro)		ALLN (Fig. 3A) and MG132 (Fig. 3B) (10 mg/mL = 21mM) reduced the cytokine-mediated NFkB activation.	
Arlt et al. 2001	MG-132		human (in vitro)		In all cell lines, gliotoxin, MG132 (10 mM) or sulfasalazine strongly reduced VP16-induced NF-kB-driven luciferase expression.	
Ortiz-Lazareno et al. 2008	MG-132		human (in vitro)		The increase in NF-kB activation induced by LPS+PMA diminished significantly from 3.27-fold to 0.94-fold in the group treated with MG132(10 mM) and later stimulated with LPS+PMA ($P < 0.002$). The activation of NF-kB induced by LPS+PMA was blocked by MG132.	
Yu and Malek 2001	MG-132		mice (in vitro)			MG132 (50mM) stabilized IL- phosphorylated STAT5, whic after 2 h in culture (Fig. 5A, la
Wang et al. 2011	MG-132		human (in vitro)			CMV-specific cytotoxicity of C decreased in the presence of
Ohkusu-Tsukada et al. 2018	MG-132	repeatedly i.p. injected 200 nmol of MG132 on days 0, 3, 5, 7, 9, 11, 13, 15, 17, and 19.	mice (in vivo)			In vivo MG132 administration DNFB-induced dermatitis red maintained the level of Th1 c alleviation of dermatitis lesion serum IgE hyperproduction a
Satou et al. 2004	bortezomib		human (in vitro, in vivo)			potently inhibits the growth of cells both in vivo and in vitro
Orciuolo et al. 2007	bortezomib	0.1 mM, 1 mM, 10 mM	human (in vitro)			the percentage of CD69/TNF with the increment of bortezo
Matsumoto et al. 2005	dehydroxymethylepoxyquinomicin (DHMEQ)		human		The addition of DHMEQ (10 mg/mL) completely inhibited the activated NF-KB for at least 8 hours.	
Nishioka et al. 2008	dehydroxymethylepoxyquinomicin (DHMEQ)		human (in vitro)		DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF-kB in Jurkat cells via inhibition of degradation of IkBa.	Exposure of PBMC to PHA gr expression of IFN-g, IL-2 and TNF-a (Fig these cells with DHMEQ (1 m reduced PHA-stimulated expression o (Fig. 3a). Similarly, PHA incre and IFN-c in Jurkat cells and cells with DHMEQ (1 mg/ml) by approximately half (Fig. 3b
Alessiani et al. 1991	FK 506		human			Five of eight deaths were due Overall, 50% of patients deve 38% suffered severe ones.
Fung et al. 1991	FK 506		human			The incidence of serious infe of FK 506, has not appeared incidence of serious infection seen in a historical group of p is that the incidence of cytom not appear to be increased w patients on CyA.
Ekberg et al. 2007	cyclosporine		human			The most commonly reported were cytomegalovirus (CMV) infection and lymphocele (Tal patients with opportunistic inf serious) was also similar amo

						cytomegalovirus infection was opportunistic infection (Table
Guler et al. 2011	i) IL-1RI$^{-/-}$ ii) Autologous Qb virus-like particle-based vaccines against IL-1a and IL-1b	ii) immunized s.c. three times before (at week: –5, –3 and –1) and once at mice week 10 post-infection				
Parnet et al. 2003	IL-1RI$^{-/-}$				Activation of NFkB in response to IL-1b was no longer apparent in IL-1RI knockout mice, confirming that this receptor is essential for the transduction of IL-1 signal in the pituitary,	
Yamada et al. 2001	NF-kB p50$^{-/-}$	knockout mice	mice			
Weih et al. 1995	RelB$^{-/-}$	knockout mice	mice			RelB-deficient animals also h immunity, as observed in con experiments.
Lin et al. 2015	Secreted IL-1α expression		mice			Both the percent and number CD8+ T cells, and CD69+ CD the expression of secreted IL
Nambu et al. 2006	IL-1a$^{-/-}$, IL-1b$^{-/-}$, IL-1a/b$^{-/-}$	knockout mice	mice			IL-1b, but not IL-1a, is require T cell activation and the induc inflammation in the delayed-t 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.

References

Alessiani, M., Kusne, S., Martin, M., Jain, A., Abu-Elmagd, K., Moser, J., Todo, S., Fung, J., Starzl, T., 1991. Infections in adult liver transplant patients under FK 506 immunosuppression. Transplant Proc 23, 1501-1503.

Arlt, A., Vorndamm, J., Breitenbroich, M., Folsch, U.R., Kalthoff, H., Schmidt, W.E., Schafer, H., 2001. Inhibition of NF-kappaB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide (VP16) or doxorubicin. Oncogene 20, 859-868.

Bent, R., Moll, L., Grabbe, S., Bros, M., 2018. Interleukin-1 Beta-A Friend or Foe in Malignancies? Int J Mol Sci 19.

Cassidy, J.P., R.: Laxer, R.; Lindsley, C, 2010. Textbook of Pediatric Rheumatology

6th Edition. Saunders.

De Benedetti, F., Gattorno, M., Anton, J., Ben-Chetrit, E., Frenkel, J., Hoffman, H.M., Kone-Paut, I., Lachmann, H.J., Ozen, S., Simon, A., Zeft, A., Calvo Penades, I., Moutschen, M., Quartier, P., Kasapcopur, O., Shcherbina, A., Hofer, M., Hashkes, P.J., Van der Hilst, J., Hara, R., Bujan-Rivas, S., Constantin, T., Gul, A., Livneh, A., Brogan, P., Cattalini, M., Obici, L., Lheritier, K., Speziale, A., Junge, G., 2018. Canakinumab for the Treatment of Autoinflammatory Recurrent Fever Syndromes. N Engl J Med 378, 1908-1919.

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

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

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

Ekberg, H., Grinyo, J., Nashan, B., Vanrenterghem, Y., Vincenti, F., Voulgari, A., Truman, M., Nasmyth-Miller, C., Rashford, M., 2007. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. Am J Transplant 7, 560-570.

Fleischmann, R.M., Schechtman, J., Bennett, R., Handel, M.L., Burmester, G.R., Tesser, J., Modafferi, D., Poulakos, J., Sun, G., 2003. Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: A large, international, multicenter, placebo-controlled trial. Arthritis Rheum 48, 927-934.

Fung, J.J., Todo, S., Tzakis, A., Alessiani, M., Abu-Elmagd, K., Jain, A., Bronster, O., Martin, M., Gordon, R., Starzl, T.E., 1991. Current status of FK 506 in liver transplantation. Transplant Proc 23, 1902-1905.

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

Guler, R., Parihar, S.P., Spohn, G., Johansen, P., Brombacher, F., Bachmann, M.F., 2011. Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. Vaccine 29, 1339-1346.

Handa, P., Vemulakonda, A., Kowdley, K.V., Uribe, M., Mendez-Sanchez, N., 2016. Mitochondrial DNA from hepatocytes as a ligand for TLR9: Drivers of nonalcoholic steatohepatitis? World J Gastroenterol 22, 6965-6971.

Hellerbrand, C., Jobin, C., Iimuro, Y., Licato, L., Sartor, R.B., Brenner, D.A., 1998. Inhibition of NFkappaB in activated rat hepatic stellate cells by proteasome inhibitors and an IkappaB super-repressor. Hepatology 27, 1285-1295.

Hoffman, H.M., Throne, M.L., Amar, N.J., Sebai, M., Kivitz, A.J., Kavanaugh, A., Weinstein, S.P., Belomestnov, P., Yancopoulos, G.D., Stahl, N., Mellis, S.J., 2008. Efficacy and safety of rilonacept (interleukin-1 Trap) in patients with cryopyrin-associated periodic syndromes: results from two sequential placebo-controlled studies. Arthritis Rheum 58, 2443-2452.

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

Imagawa, T., Nishikomori, R., Takada, H., Takeshita, S., Patel, N., Kim, D., Lheritier, K., Heike, T., Hara, T., Yokota, S., 2013. Safety and efficacy of canakinumab in Japanese patients with phenotypes of cryopyrin-associated periodic syndrome as established in the first open-label, phase-3 pivotal study (24-week results). Clin Exp Rheumatol 31, 302-309.

Issafras, H., Corbin, J.A., Goldfine, I.D., Roell, M.K., 2014. Detailed mechanistic analysis of gevokizumab, an allosteric anti-IL-1beta antibody with differential receptor-modulating properties. J Pharmacol Exp Ther 348, 202-215.

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

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat Rev Immunol 16, 626-638.

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

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

Lee, K.M., Kang, B.S., Lee, H.L., Son, S.J., Hwang, S.H., Kim, D.S., Park, J.S., Cho, H.J., 2004. Spinal NF-kB activation induces COX-2 upregulation and contributes to inflammatory pain hypersensitivity. Eur J Neurosci 19, 3375-3381.

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

Lin, D., Lei, L., Zhang, Y., Hu, B., Bao, G., Liu, Y., Song, Y., Liu, C., Wu, Y., Zhao, L., Yu, X., Liu, H., 2015. Secreted IL-1alpha promotes T-cell activation and expansion of CD11b(+) Gr1(+) cells in carbon tetrachloride-induced liver injury in mice. Eur J Immunol 45, 2084-2098.

Mansouri, B., Richards, L., Menter, A., 2015. Treatment of two patients with generalized pustular psoriasis with the interleukin-1beta inhibitor gevokizumab. Br J Dermatol 173, 239-241.

Matsumoto, G., Namekawa, J., Muta, M., Nakamura, T., Bando, H., Tohyama, K., Toi, M., Umezawa, K., 2005. Targeting of nuclear factor kappaB Pathways by dehydroxymethylepoxyquinomicin, a novel inhibitor of breast carcinomas: antitumor and antiangiogenic potential in vivo. Clin Cancer Res 11, 1287-1293.

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

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

Newton, K., Dixit, V.M., 2012. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol 4.

Nishioka, C., Ikezoe, T., Jing, Y., Umezawa, K., Yokoyama, A., 2008. DHMEQ, a novel nuclear factor-kappaB inhibitor, induces selective depletion of alloreactive or phytohaemagglutinin-stimulated peripheral blood mononuclear cells, decreases production of T helper type 1 cytokines, and blocks maturation of dendritic cells. Immunology 124, 198-205.

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

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

Ortiz-Lazareno, P.C., Hernandez-Flores, G., Dominguez-Rodriguez, J.R., Lerma-Diaz, J.M., Jave-Suarez, L.F., Aguilar-Lemarroy, A., Gomez-Contreras, P.C., Scott-Algara, D., Bravo-Cuellar, A., 2008. MG132 proteasome inhibitor modulates proinflammatory cytokines production and expression of their receptors in U937 cells: involvement of nuclear factor-kappaB and activator protein-1. Immunology 124, 534-541.

Palombella, V.J., Rando, O.J., Goldberg, A.L., Maniatis, T., 1994. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 78, 773-785.

Parnet, P., Pousset, F., Laye, S., 2003. NF kappa B activation in mouse pituitary: comparison of response to interleukin-1 beta and lipopolysaccharide. J Neuroendocrinol 15, 304-314.

Roell, M.K., Issafras, H., Bauer, R.J., Michelson, K.S., Mendoza, N., Vanegas, S.I., Gross, L.M., Larsen, P.D., Bedinger, D.H., Bohmann, D.J., Nonet, G.H., Liu, N., Lee, S.R., Handa, M., Kantak, S.S., Horwitz, A.H., Hunter, J.J., Owyang, A.M., Mirza, A.M., Corbin, J.A., White, M.L., 2010. Kinetic approach to pathway attenuation using XOMA 052, a regulatory therapeutic antibody that modulates interleukin-1beta activity. J Biol Chem 285, 20607-20614.

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

Schlesinger, N., Alten, R.E., Bardin, T., Schumacher, H.R., Bloch, M., Gimona, A., Krammer, G., Murphy, V., Richard, D., So, A.K., 2012. Canakinumab for acute gouty arthritis in patients with limited treatment options: results from two randomised, multicentre, active-controlled, double-blind trials and their initial extensions. Ann Rheum Dis 71, 1839-1848.

Settas, L.D., Tsimirikas, G., Vosvotekas, G., Triantafyllidou, E., Nicolaides, P., 2007. Reactivation of pulmonary tuberculosis in a patient with rheumatoid arthritis during treatment with IL-1 receptor antagonists (anakinra). J Clin Rheumatol 13, 219-220.

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

Sigma-Aldrich, IL-1Ra Specification Sheet.

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

Vallejo, S., Palacios, E., Romacho, T., Villalobos, L., Peiro, C., Sanchez-Ferrer, C.F., 2014. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. Cardiovasc Diabetol 13, 158.

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

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

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

Weih, F., Carrasco, D., Durham, S.K., Barton, D.S., Rizzo, C.A., Ryseck, R.P., Lira, S.A., Bravo, R., 1995. Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. Cell 80, 331-340.

Yamada, H., Mizumo, S., Horai, R., Iwakura, Y., Sugawara, I., 2000. Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. Lab Invest 80, 759-767.

Yamada, H., Mizuno, S., Reza-Gholizadeh, M., Sugawara, I., 2001. Relative importance of NF-kappaB p50 in mycobacterial infection. Infect Immun 69, 7100-7105.

Yang, Han, Z., Oppenheim, J.J., 2017. Alarmins and immunity. Immunol Rev 280, 41-56.

Yokota, S., Imagawa, T., Nishikomori, R., Takada, H., Abrams, K., Lheritier, K., Heike, T., Hara, T., 2017. Long-term safety and efficacy of canakinumab in cryopyrin-associated periodic syndrome: results from an open-label, phase III pivotal study in Japanese patients. Clin Exp Rheumatol 35 Suppl 108, 19-26.

Yu, A., Malek, T.R., 2001. The proteasome regulates receptor-mediated endocytosis of interleukin-2. J Biol Chem 276, 381-385.

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

Biological Context

Level of Biological Organization

Level of Biological Organization

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).

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

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

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

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

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

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 PRPs 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 PRPs, the signaling through TLR4 is best characterized. In addition, it is beyond the scope of this AOP to cover all signaling through each PRP. 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). 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 (Mucke, 2012). Epigalocathechin gallate (EGCG) has been reported to inhibit NF-κB activation through inhibition of p65 phosphorylation (Wheeler et al., 2004).

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-1b production, minocycline, and two prodrugs, pralnacasan (VX-740) and belnacasan (VX-765) that are orally absorbed and converted into the active principle, VRT-018858 and VRT-043198, respectively (Fenini 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 (anti-IL-1β antibody) (Church and McDermott, 2009).

How it is Measured or Detected

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

References

Alten, R., Gram, H., Joosten, L.A., van den Berg, W.B., Sieper, J., Wassenberg, S., Burmester, G., van Riel, P., Diaz-Lorente, M., Bruin, G.J., Woodworth, T.G., Rordorf, C., Batard, Y., Wright, A.M., Jung, T., 2008. The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. Arthritis Res Ther 10, R67.

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

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

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

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

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

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

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

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

Jeon, Y.J., Han, S.H., Lee, Y.W., Lee, M., Yang, K.H., Kim, H.M., 2000. Dexamethasone inhibits IL-1 beta gene expression in LPS-stimulated RAW 264.7 cells by blocking NF-kappa B/Rel and AP-1 activation. Immunopharmacology 48, 173-183.

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

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat Rev Immunol 16, 626-638.

Lee, K.L., Ambler, C.M., Anderson, D.R., Boscoe, B.P., Bree, A.G., Brodfuehrer, J.I., Chang, J.S., Choi, C., Chung, S., Curran, K.J., Day, J.E., Dehnhardt, C.M., Dower, K., Drozda, S.E., Frisbie, R.K., Gavrin, L.K., Goldberg, J.A., Han, S., Hegen, M., Hepworth, D., Hope, H.R., Kamtekar, S., Kilty, I.C., Lee, A., Lin, L.L., Lovering, F.E., Lowe, M.D., Mathias, J.P., Morgan, H.M., Murphy, E.A., Papaioannou, N., Patny, A., Pierce, B.S., Rao, V.R., Saiah, E., Samardjiev, I.J., Samas, B.M., Shen, M.W.H., Shin, J.H., Soutter, H.H., Strohbach, J.W., Symanowicz, P.T., Thomason, J.R., Trzupek, J.D., Vargas, R., Vincent, F., Yan, J., Zapf, C.W., Wright, S.W., 2017. Discovery of Clinical Candidate 1-{[(2S,3S,4S)-3-Ethyl-4-fluoro-5-oxopyrrolidin-2-yl]methoxy}-7-methoxyisoquinoli ne-6-carboxamide (PF-06650833), a Potent, Selective Inhibitor of Interleukin-1 Receptor Associated Kinase 4 (IRAK4), by Fragment-Based Drug Design. J Med Chem 60, 5521-5542.

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

McGuire, V.A., Ruiz-Zorrilla Diez, T., Emmerich, C.H., Strickson, S., Ritorto, M.S., Sutavani, R.V., Weibeta, A., Houslay, K.F., Knebel, A., Meakin, P.J., Phair, I.R., Ashford, M.L., Trost, M., Arthur, J.S., 2016. Dimethyl fumarate blocks pro-inflammatory cytokine production via inhibition of TLR induced M1 and K63 ubiquitin chain formation. Sci Rep 6, 31159.

McIntyre, K.W., Stepan, G.J., Kolinsky, K.D., Benjamin, W.R., Plocinski, J.M., Kaffka, K.L., Campen, C.A., Chizzonite, R.A., Kilian, P.L., 1991. Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 173, 931-939.

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

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

Newton, K., Dixit, V.M., 2012. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol 4.

Peng, H., Guerau-de-Arellano, M., Mehta, V.B., Yang, Y., Huss, D.J., Papenfuss, T.L., Lovett-Racke, A.E., Racke, M.K., 2012. Dimethyl fumarate inhibits dendritic cell maturation via nuclear factor kappaB (NF-kappaB) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) and mitogen stress-activated kinase 1 (MSK1) signaling. J Biol Chem 287, 28017-28026.

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

Quartier, P., 2011. Interleukin-1 antagonists in the treatment of autoinflammatory syndromes, including cryopyrin-associated periodic syndrome. Open Access Rheumatol 3, 9-18.

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

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

Stack, J.H., Beaumont, K., Larsen, P.D., Straley, K.S., Henkel, G.W., Randle, J.C., Hoffman, H.M., 2005. IL-converting enzyme/caspase-1 inhibitor VX-765 blocks the hypersensitive response to an inflammatory stimulus in monocytes from familial cold autoinflammatory syndrome patients. J Immunol 175, 2630-2634.

Vallabhapurapu, S., Karin, M., 2009. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 27, 693-733.

van Furth, A.M., Seijmonsbergen, E.M., Langermans, J.A., van der Meide, P.H., van Furth, R., 1995. Effect of xanthine derivates and dexamethasone on Streptococcus pneumoniae-stimulated production of tumor necrosis factor alpha, interleukin-1 beta (IL-1 beta), and IL-10 by human leukocytes. Clin Diagn Lab Immunol 2, 689-692.

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

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

Wang, Y., Tang, Q., Duan, P., Yang, L., 2018. Curcumin as a therapeutic agent for blocking NF-kappaB activation in ulcerative colitis. Immunopharmacol Immunotoxicol 40, 476-482.

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

Wheeler, D.S., Catravas, J.D., Odoms, K., Denenberg, A., Malhotra, V., Wong, H.R., 2004. Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. J Nutr 134, 1039-1044.

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

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).

Key Event Description

The NF-kB pathway consists of a series of events where the transcription factors of the NF-kB family play the key role. The NF-κB pathway can be activated by a range of stimuli, including TNF receptor activation by TNF-a, or IL-1R1 activation by IL-1a or b. Upon pathway activation, the IKK complex will be phosphorylated, which in turn phosphorylates IkBa. This NF-kB inhibitor will be K48-linked ubiquitinated and degradated, allowing NF-kB to translocate to the nucleus. There, this transcription factor can express pro-inflammatory and anti-apoptotic genes. Furthermore, negative feedback genes are also transcribed and include IkBa and A20. When the NF-kB pathway is inhibited, its translocation will be delayed (or absent), resulting in less or no regulation of NF-kB target genes. This can be achieved by IKK inhibitors, proteasome inhibitors, nuclear translocation inhibitors or DNA-binding inhibitors. (Frederiksson 2012)(Gupta et al. 2010)(Huppelschoten 2017)(Liu et al. 2017). Therefore, inhibition of IL-1R1 activation suppresses activation of NF-kB.

How it is Measured or Detected

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

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

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

NF-κB p65 (Total/Phospho) ELISA

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

References

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

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

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

Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat Rev Immunol 16, 626-638.

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

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

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

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

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

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

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.

References

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

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

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

List of Adverse Outcomes in this AOP

Event: 986: Increase, Increased susceptibility to infection

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. (Fleischmann et al., 2003; De Benedetti et al., 2018; Hirsch et al., 1996)

Key Event Description

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

How it is Measured or Detected

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

Regulatory Significance of the AO

After L-1R antagonist or neutralizing antibody such as IL-1Ra (generic anakinra), canakinumab (anti-IL-1b antibody) and rilonacept (soluble IL-1R) became available to treat some of autoinflammatory syndromes, it became clear that these inhibitors increased the frequency of serious bacterial infection (De Benedetti et al., 2018; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Migkos et al., 2015; Schlesinger et al., 2012; Yokota et al., 2017).

References

Auphan, N., DiDonato, J.A., Rosette, C., et al., 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270, 286-290.

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

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

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

Guler, R., Parihar, S.P., Spohn, G., et al., 2011. Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. Vaccine 29, 1339-1346.

Horino, T., Matsumoto, T., Ishikawa, H., et al., 2009. Interleukin-1 deficiency in combination with macrophage depletion increases susceptibility to Pseudomonas aeruginosa bacteremia. Microbiol Immunol 53, 502-511.

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

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

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

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.

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

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

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

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

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

Appendix 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

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

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

Evidence Supporting this KER

Biological Plausibility

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).

Empirical Evidence

IL-1Ra blocks IL-1 signaling:

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

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

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

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

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

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

Canakinumab (ACZ885, Ilaris) blocks IL-1 signaling

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

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

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

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

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

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).

NF-κB inbitiors

Quite a few compounds have been reported to inhibit NF-κB signaling by several different mechanisms reviewed by Fuchs (Fuchs 2010). Several studies have shown intriguing pharmacologic effects associated with curcumin, which inhibits NF-κB expression by regulating NF-κB/IkB pathway and down-regulation expression of pro-inflammatory cytokines, such as Interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α (Wang et al. 2018).

Chemicals	Target and Function
AS602868 (anilino-pyrimidine derivative)	IKK inhibitor, inhibitor of NF-KB nuclear translocation and induc- tion of apoptosis
AT514 (a cyclic depsipeptide)	AKT/NF-KB inhibitor
Bay117082	Mitochondrial dysfunction and apoptosis
Berbamine	Upregulation of A20, downregulation of IKKa and inhibition of p65 nuclear translocation
BMS-345541	IKB kinase inhibitor
Bortezomib	Proteasome inhibition, stabilization of IKB
Carfilzomib (PR-171)	Proteasome inhibition, stabilization of IKB
Celastrol	IKB kinase inhibitor, inhibitor of NF-KB expression on both, the pro- tein and mRNA level
CEP-18770	Proteasome inhibition, stabilization of IKB
Curcumin	Inhibitor of NF-KB activation, induces G1/S arrest and induces apop- tosis

DHMEQ	Inhibitor of both canonical and non-canonical NF-KB activating pathways at the level of nuclear translocation
Epicatechin	Inhibitor of NF-KB binding to DNA
Etodolac (SDX-101)	Antiinflammatory and prostaglandin synthase inhibition
Flavopiridol	IKKa inhibitor
Kamebakaurin	Inhibitor of p50 binding to DNA
LC-1 (dimethylaminoparthenolide, DMAPT)	Inhibition of RelA binding to DNA
MG132 (peptidyl aldehyde of tri-leucine)	Proteasome inhibition, stabilization of IKB, mitochondrial dysfunction and apoptosis
MLN120B (-carboline derivative)	IKK inhibitor
PS-1145 (-carboline derivative)	IKK inhibitor
Salinosporamide A (NPI-0052 or ML858)	Proteasome inhibition,stabilization of IKB, mitochondrial dysfunction and apoptosis
SDX-308 (CEP-180802)	Caspase activation, poly (ADP-ribose) polymerase cleavage and apoptosis
SN50 (cell-permeable inhibitor peptide)	NF-KB nuclear translocation inhibitor
Triptolide	ROS generation,caspase activation and apoptosis
Xanthohumol	AKT/NF-KB inhibitor
4-hydroxy-2-nonenal	Reduction of IKBa mRNA levels and decrease in phosphorylated IKBa

Quantitative Understanding of the Linkage

See Empirical Evidence.

Response-response relationship

IL-1Ra blocks IL-1 signaling:

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

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

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

References

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

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

Brikos, C., Wait, R., Begum, S., et al., 2007. Mass spectrometric analysis of the endogenous type I interleukin-1 (IL-1) receptor signaling complex formed after IL-1 binding identifies IL-1RAcP, MyD88, and IRAK-4 as the stable components. Mol Cell Proteomics 6, 1551-1559.

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

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

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

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

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

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

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

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

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

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.

Lye, E., Dhanji, S., Calzascia, T., Elford, AR., Ohashi, PA.. 2008. IRAK-4 kinase activity is required for IRAK-4-dependent innate and adaptive immune responses. Eur J Immunol. 38: 870-876/

Lye, E., Mirtos, C., Suzuki, N., Suzuki, S., Yeh, W-C. 2004. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. J Biol Chem. 279: 40653-40658.

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

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

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

Shuck, M.E., Eessalu, T.E., Tracey, D.E., et al., 1991. Cloning, heterologous expression and characterization of murine interleukin 1 receptor antagonist protein. Eur J Immunol 21, 2775-2780.

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

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

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

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

Evidence Supporting this KER

Biological Plausibility

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

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

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

Empirical Evidence

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

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

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

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

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

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

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

Quantitative Understanding of the Linkage

A representative NF-kB inhibitor, MG132 that suppresses NF-kB activity at more than 10 mM (Fiedler et al. 1998) suppresses IL-2-induced activation of STAT5 at 50 mM. (Yu and Malek 2001)
A representative NF-kB inhibitor, DHMEQ (1mg/mL) blocked PHA-induced nuclear translocation of NF-kB in Jurkat cells via inhibition of degradation of IkBa. Preincubation of peripheral blood mononuclear cells with DHMEQ (1 mg/ml, 3 hr) greatly reduced PHA-stimulated expression of IFN-g, IL-2 and TNF-a genes.

Response-response relationship

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

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

References

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

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

Nishioka, C., Ikezoe, T., Jing, Y., Umezawa, K., Yokoyama, A., 2008. DHMEQ, a novel nuclear factor-kappaB inhibitor, induces selective depletion of alloreactive or phytohaemagglutinin-stimulated peripheral blood mononuclear cells, decreases production of T helper type 1 cytokines, and blocks maturation of dendritic cells. Immunology 124, 198-205.

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

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

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

Pordanjani, S.M., Hosseinimehr, S.J., 2016. The Role of NF-kB Inhibitors in Cell Response to Radiation. Curr Med Chem 23, 3951-3963.

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

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

Weih, F., Carrasco, D., Durham, S.K., Barton, D.S., Rizzo, C.A., Ryseck, R.P., Lira, S.A., Bravo, R., 1995. Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. Cell 80, 331-340.

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	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

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

Evidence Supporting this KER

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

Biological Plausibility

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

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

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

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

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

Empirical Evidence

Administration of IL-1R antagonist or neutralizing antibody such as IL-1Ra (generic anakinra), canakinumab (anti-IL-1β antibody) and rilonacept (soluble IL-1R) led to the suppression of downstream phenomena, which included internalization of IL-1 (Dripps et al., 1991), production of PGE_2 (Hannum et al., 1990; Seckinger et al., 1990), IL-6 (Goh et al., 2014), and T cell proliferation (Seckinger et al., 1990).

Since these inhibitors became available to treat some of autoinflammatory syndromes, it became clear that these inhibitors increased the frequency of serious bacterial infection (De Benedetti et al., 2018; Genovese et al., 2004; Imagawa et al., 2013; Kullenberg et al., 2016; Lachmann et al., 2009; Lequerre et al., 2008; Migkos et al., 2015; Schlesinger et al., 2012; Yokota et al., 2017).

References

Auphan, N., DiDonato, J.A., Rosette, C., et al., 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270, 286-290.

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

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

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

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

Goh, A.X., Bertin-Maghit, S., Ping Yeo, S., et al., 2014. A novel human anti-interleukin-1beta neutralizing monoclonal antibody showing in vivo efficacy. MAbs 6, 765-773.

Guler, R., Parihar, S.P., Spohn, G., et al., 2011. Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. Vaccine 29, 1339-1346.

Hannum, C.H., Wilcox, C.J., Arend, W.P., et al., 1990. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 343, 336-340.

Horino, T., Matsumoto, T., Ishikawa, H., et al., 2009. Interleukin-1 deficiency in combination with macrophage depletion increases susceptibility to Pseudomonas aeruginosa bacteremia. Microbiol Immunol 53, 502-511.

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

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

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

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.

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

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

Seckinger, P., Kaufmann, M.T., Dayer, J.M., 1990. An interleukin 1 inhibitor affects both cell-associated interleukin 1-induced T cell proliferation and PGE2/collagenase production by human dermal fibroblasts and synovial cells. Immunobiology 180, 316-327.

Soares, M.P., Teixeira, L., Moita, L.F., 2017. Disease tolerance and immunity in host protection against infection. Nat Rev Immunol 17, 83-96.

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

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

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