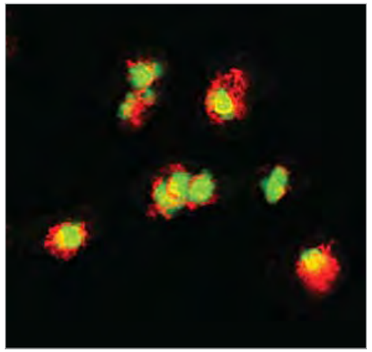
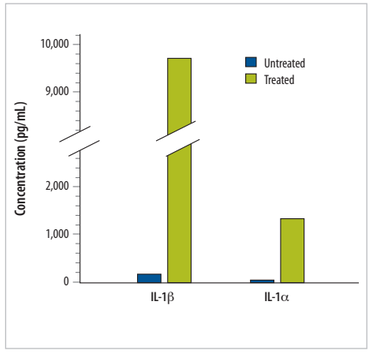
Hereditary Auto-Inflammatory Diseases

Disease	Mutated Protein	Pattern of Inheritance
Blau Syndrome	CARD15	Autosomal Dominant
Familial Cold Auto-inflammatory Syndrome (FCAS)	NALP3 (Cryopyrin)	Autosomal Dominant
Familial Mediterranean Fever (FMF)	Pyrin	Autosomal Recessive
Hyperimmunoglobulinemia D with Periodic Fever Syndrome (HIDS)	Mevalonate Kinase	Autosomal Recessive
Muckle-Wells Syndrome (MWS)	NALP3 (Cryopyrin)	Autosomal Dominant
Neonatal-Onset Multisystem Inflammatory Disease (NOMID) or Chronic Infantile Neurologic Cutaneous Articular Syndrome (CINCA)	NALP3 (Cryopyrin)	Autosomal Dominant
Pyogenic Sterile Arthritis, Pyoderma Gangrenosum, Acne Syndrome (PAPA)	CD2-binding protein 1	Autosomal Dominant
Tumor Necrosis Factor Receptor-Associated Periodic Syndrome (TRAPS)	TNF RI	Autosomal Dominant

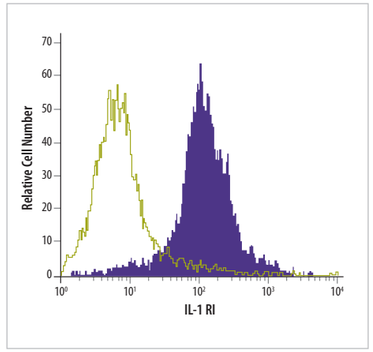
Mutated Proteins and Patterns of Inheritance Associated with Hereditary Auto-inflammatory Diseases.
Hereditary auto-inflammatory diseases are caused by autosomal recessive or autosomal dominant mutations that primarily affect proteins involved in the regulation of cytokine processing, specifically IL-1β, IL-18, and IL-33. NALP3 (Cryopyrin) mutations lead to constitutive inflammasome activation, processing of pro-caspase-1, and the overproduction of IL-1β. Mutations in pyrin, or CD2-binding protein, a protein that interacts with pyrin, also allow increased IL-1β production, due to a defect in caspase-1 inhibition. The mechanism by which mutations in mevalonate kinase, TNF RI, and CARD15 cause auto-inflammatory disorders are not currently well understood, although CARD15 has a NACHT domain similar to that found in NALP3.



Detection of IL-1β in Con A-activated Mouse Splenocytes. Mouse splenocytes were activated with Con A. IL-1β was detected by indirect immunofluorescent staining using anti-mouse IL-1β/IL-1F2 polyclonal antibody (Catalog # AF401-NA) followed by Rhodamine Red™ anti-goat secondary antibody. Cells were counterstained using FluoroNissl™ Green (green fluorescence).



Measurement of IL-1α and IL-1β in Peripheral Blood Mononuclear Cell Supernatants. Human peripheral blood mononuclear cells were treated with PHA. The levels of IL-1α and IL-1β in cell supernatants from treated and non-treated cells were assessed using the IL-1α/IL-1F1 Quantikine® ELISA Kit (Catalog # DLA50; 5 x 10^6 cells/mL) or the IL-1β/IL-1F2 QuantiGlo® ELISA Kit (Catalog # QLB00; 1 x 10^6 cells/mL).



Detection of IL-1 RI in Mouse Splenocytes by Flow Cytometry. Mouse $B220^+$ splenocytes were stained with carboxyfluorescein (CFS)-conjugated anti-mouse IL-1 RI monoclonal antibody (Catalog # FAB7712F; filled histogram) or CFS-conjugated rat IgG_{2B} isotype control antibody (Catalog # IC013F; open histogram).

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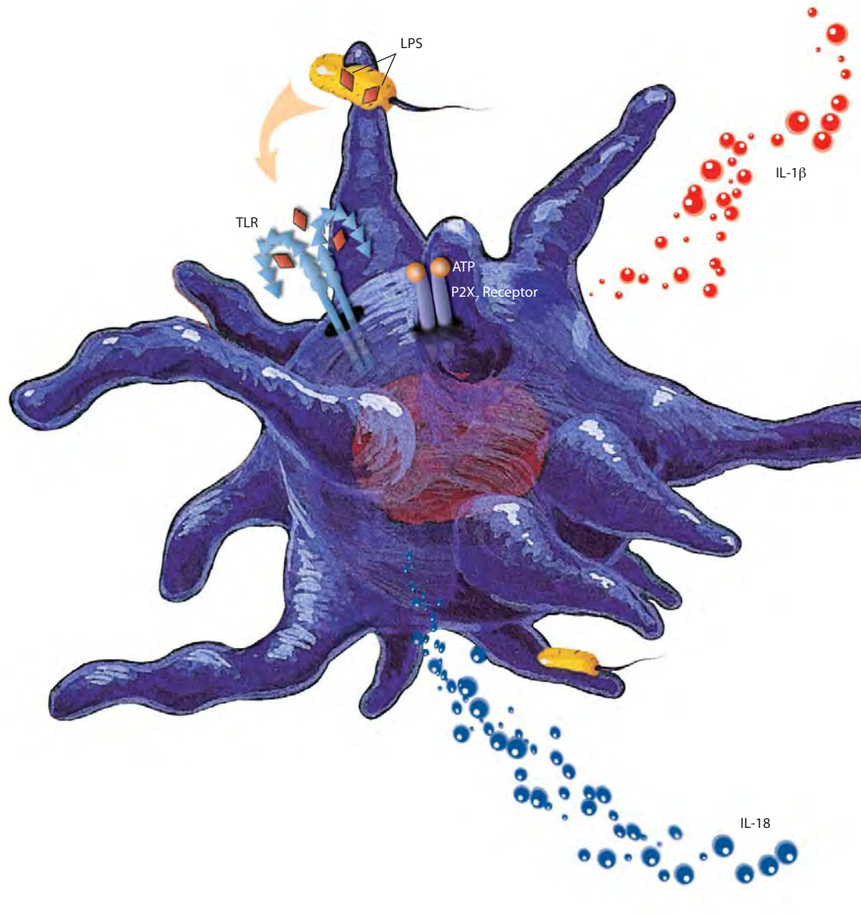


The Role of the NALP3 Inflammasome, Caspase-1, & Cytokine Secretion in Auto-Inflammatory Diseases

Auto-inflammatory diseases are characterized by recurrent attacks of fever and localized systemic inflammation that arise in the absence of pathogen-induced infections. Many of these diseases, including Muckle-Wells Syndrome (MWS), Familial Cold Auto-inflammatory Syndrome (FCAS), and Neonatal-Onset Multisystem Inflammatory Disease (NOMID), also known as Chronic Infantile Neurologic Cutaneous Articular Syndrome (CINCA), are caused by mutations in the CIAS1 gene which encodes a protein called cryopyrin or NALP3. NALP3 associates with pro-caspase-1 by way of an adaptor protein, ASC (apoptosis-associated speck-like protein with a caspase recruitment domain), to form the NALP3 inflammasome. Formation of this complex leads to the activation of pro-caspase-1 and the subsequent processing and secretion of pro-inflammatory cytokines, such as IL-1β and IL-18. Autosomal dominant mutations in NALP3, like those found in MWS, FCAS, or NOMID, are thought to result in a constitutively active inflammasome leading to spontaneous caspase-1 activation, and the overproduction of IL-1β.

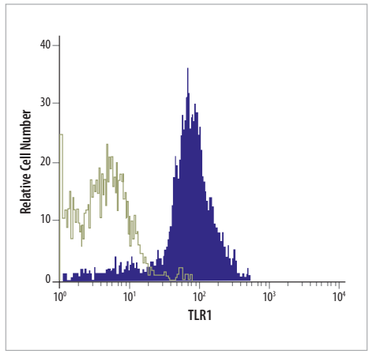
Although cytokine secretion is normally required to combat infection, unprovoked or sustained secretion can have detrimental effects that are associated with the pathogenesis of auto-inflammatory diseases. Therefore, clinical approaches to treat the symptoms of many auto-inflammatory diseases are directed at reducing the activity of IL-1β. Intrinsic mechanisms that neutralize the effects of IL-1β in response to pathogen-induced cytokine release, such as decoy or soluble receptors that sequester IL-1β, or non-signaling antagonists that compete with IL-1β for receptor-binding, have provided a guide for the design of drugs targeting IL-1β. Anakinra, a recombinant IL-1 receptor antagonist, is being used to successfully treat the symptoms associated with many auto-inflammatory disorders.

Current evidence suggests that different pathogens trigger the oligomerization of distinct inflammasome complexes that differ in the sensor and adaptor proteins that they contain. Further characterization of these complexes and the mechanisms by which they are activated will lead to a greater understanding of how the innate immune system protects against invading pathogens and the defects associated with auto-inflammatory diseases.

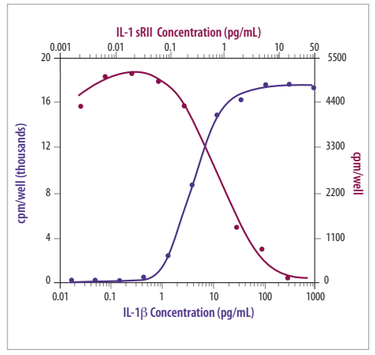
R&D Systems offers a wide range of research reagents useful for the study of inflammasome complexes and auto-inflammation.

References

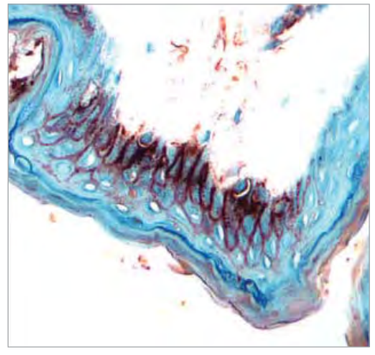
1. Martinon, F. & J. Tschopp (2004) Cell **117**:561.
2. Tunca, M. & H. Ozdogan (2005) Curr. Drug Targets Inflamm. Allergy **4**:77.
3. Sutterwala, F.S. *et al.* (2006) Immunity **24**:317.



Detection of TLR1 in Human Monocytes by Flow Cytometry. Human monocytes were stained with allophycocyanin (APC)-conjugated anti-human TLR1 polyclonal antibody (Catalog # FAB1484A; filled histogram) or APC-conjugated goat IgG control antibody (Catalog # IC108A; open histogram).



Inhibition of IL-1β Activity by Soluble IL-1 RII. Recombinant human IL-1β/IL-1F2 (Catalog # 201-LB) stimulates the proliferation of murine T helper D10.G4.1 cells (purple line; measured by ^{3}H-thymidine incorporation). The stimulatory effect induced by 50 pg/mL IL-1β/IL-1F2 was inhibited in a dose-dependent manner by soluble recombinant human IL-1 RII (Catalog # 263-2R; red line).



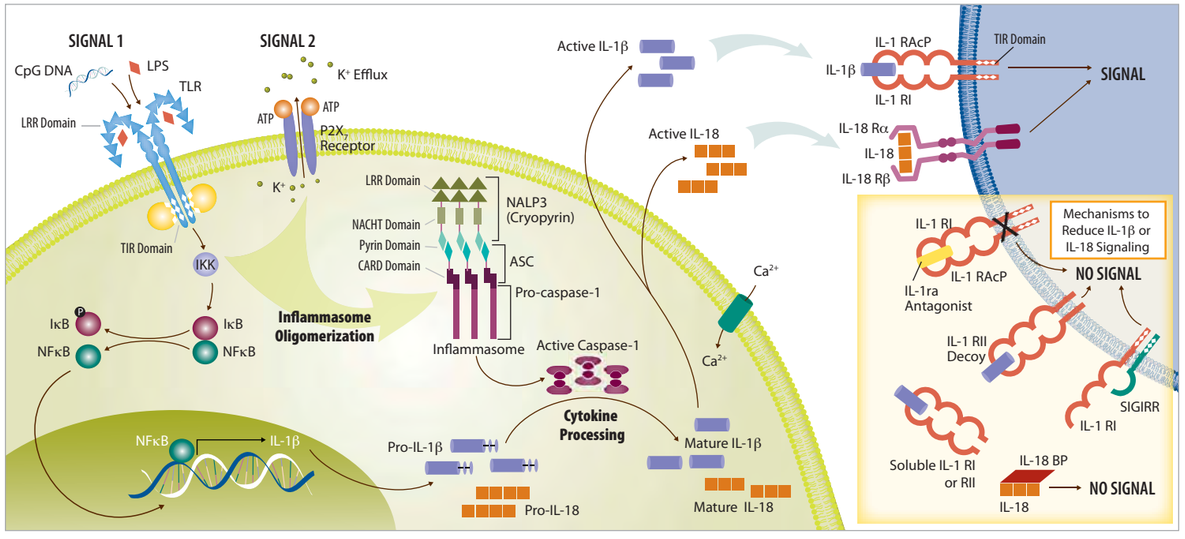
IL-18 Rα Expression in Human Skin. IL-18 Rα was detected in cryostat tissue sections of normal human skin using anti-human IL-18 Rα/IL-1 R5 polyclonal antibody (Catalog # AF840). Tissues were stained using the anti-goat HRP-AEC Cell and Tissue Staining Kit (Catalog # CTS009; red) and counterstained with hematoxylin (blue).

Inflammasome Research Products Available from R&D Systems

Molecule	Antibodies	Proteins	ELISAs/Assays
ASC	H		
Caspase-1			H Ms
Caspase-12	M R		
IL-1α/IL-1F1	H M R CR P	H M R CR P	H M R
IL-1β/IL-1F2	H M R Ca CR E F P	H M R Ca CR E F P Pr	H M R F P
IL-1β/IL-1F2 Propeptide			H
IL-1ra/IL-1F3	H M P E	H M R E P	H M
IL-1 RAcP/IL-1 R3	H	H	
IL-1 Rrp2/IL-1 R6	H M R	H M R	
IL-1 RI	H M	H M	H
IL-1 RII	H M	H M	H
IL-6	H M R Ca CR E F P	H M R Ca CR E F P	H M R Ca F P
IL-18/IL-1F4	H M R Ca P Pr	H M R P Pr	H M
IL-18/IL-1F4 Propeptide	H		
IL-18 BPa	H	H	H
IL-18 BPc	M		
IL-18 BPd	M	M	
IL-18 Rα/IL-1 R5	H M	H	

Molecule	Antibodies	Proteins	ELISAs/Assays
IL-18 Rβ/IL-1 R7	H M	H	
IL-32γ		H	
IL-33	H M	H M	H M
IL-33 Propeptide	H M		
MyD88	H M R		H
NAIP	H		
NALP2	H		
SIGIRR	H M	H M	
ST2/IL-1 R4	H M	H M	H M
TLR1	H M	M	
TLR2	H M	H M	
TLR3	H M	H M	
TLR4	H M	H	
TLR6	M	M	
TLR9	H		
TNF-α	H M R B Ca CR E P Pr	H M R B Ca CR E F P Pr	H M R Ca E F P Pr
TNF RI/TNFRSF1A	H M	H M Ca	H M

Abbreviation Key: **B**: Bovine **Ca**: Canine **CR**: Cotton Rat **E**: Equine **F**: Feline **H**: Human **M**: Mouse **Ms**: Multi-species **P**: Porcine **Pr**: Primate **R**: Rat



Activation of Pro-caspase-1 by the NALP3 Inflammasome Promotes the Secretion of IL-1β and IL-18.
The oligomerization of the NALP3 inflammasome is triggered by two signals, the recognition of pathogen-associated components, such as lipopolysaccharide (LPS) or CpG DNA by the Toll-like receptors (TLR), and an ionic perturbation in the cell, such as an efflux of potassium (K^+) caused by the ATP-dependent activation of the purinergic $P2X_7$ receptor. Oligomerization of the NALP3 inflammasome is initiated by NALP3 interacting with the ASC adaptor protein by way of its pyrin domain. ASC subsequently recruits pro-caspase-1 to the inflammasome through its caspase recruitment domain (CARD). Formation of the inflammasome leads to the cleavage and activation of pro-caspase-1 which subsequently cleaves pro-IL-1β and pro-IL-18 into mature forms that can be secreted by the cell. Cytokine secretion leads to inflammation at the site of an infection. Several intrinsic mechanisms (highlighted square) reduce the effects of IL-1β, including soluble IL-1 receptors, decoy receptors (IL-1 RII), receptor antagonists (IL-1ra), and other negative regulators such as SIGIRR (single Ig IL-1-related receptor) which prevents IL-1 RI/IL-1 RAcP heterodimerization and inhibits the recruitment of downstream signaling molecules. An IL-18 binding protein (IL-18 BP) can interact with IL-18 and prevent it from binding to its receptor. Some auto-inflammatory diseases result from the unprovoked or sustained activation of the inflammasome that leads to spontaneous caspase-1 activation and the hypersecretion of pro-inflammatory cytokines.

For more information on products for inflammasome research, please visit our website at www.RnDSystems.com/go/inflammasome