Macrophages, the Inflammasome and Interleukin-1β in Cancer

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Inflammation has been considered for a long time as being of limited duration and beneficial to any immune response. However, it is now widely accepted that smoldering chronic inflammation is a significant force driving chronic disease, including cancer, where it responds to tissue injury to promote repair through cell remodeling, proliferation and angiogenesis. Thus, chronic inflammation considerably contributes to most stages of tumorigenesis, including tumor initiation, promotion, malignant differentiation, invasion and metastasis[1]. About 18% of all cancers have a chronic infectious etiology[2,3]. This close correlation between infection, chronic local inflammation and tumorigenesis is well established for several cancers: bladder cancer is associated with Schistosoma and Gram-negative uropathogen infections; cervical cancer with Papillomavirus infection and cervicitis; ovarian cancer with Pelvic inflammatory disease; pancreatic cancer with pancreatitis; gastric cancer and MALT lymphoma with *Helicobacter pylori* infection and gastritis; esophageal cancer with Oesophagitis and Barrett's metaplasia; colorectal cancer with inflammatory bowel disease and and Bacteroides infections; hapatocellular cancer with Hepatitis virus B and C infections and hepatitis; bronchial cancer with silicosis, asbestosis, and bronchitis; mesothelioma with asbestosis; Karposi's sarcoma with Human herpes virus type 8 infection; Burkitt's lymphoma and Hodgkin's disease with Mononucleosis and Epstein-Barr virus infection; skin cancer with Papillomavirus

infection and warts; breast cancer with inflammation; and gall bladder cancer with Cholecystitis and bacterial infection[4]. While acute inflammatory responses can be beneficial for cancer treatment, chronic inflammation frequently caused by tumorigenic pathogens and autoimmunity, significantly increases one's cancer risk. In addition, solid tumors promote a pro-inflammatory microenvironment. Cancer cells recruit leukocytes, which produce tumor promoting chemokines and cytokines. Furthermore, solid tumors ultimately outgrow all available blood supply resulting in oxygen deprivation and necrosis, which is pro-inflammatory through release of inflammatory mediators, including IL-1, which perpetuate the inflammatory response.

Tumor-associated macrophages.

There is continuous interplay between immune cells and cancer cells. Macrophages are abundantly present in the tumor microenvironment, and are referred to as tumor-associated macrophages (TAMs), which are alternative activated M2 macrophages and therefore promote cancer growth, survival, metastasis and impair immunosurveillance by the adaptive immune system[5,6]. Many tumor cells produce chemokines, including M-CSF, VEGF or CCL2, to attract circulating blood monocytes to infiltrate tumor tissues and to differentiate into macrophages. While macrophages recognize and are able to eliminate cancer cells, they can be exploited to locally produce pro-angiogenic factors, growth factors or matrix metalloproteases to aid tumor cell proliferation, invasion and metastasis. TAMs promote proliferation through release of growth factors, such as EGF, M-CSF, PDGF, FGF or TGFβ. They promote angiogenesis to increase the local blood supply for tumors through the release of pro-angiogenic factors, including VEGF and chemokines, including CCL2, CCL5, CXCL1, CXCL8, CXCL13 and CXCL12. TAMs release matrix metalloproteases (MMPs), including MMP-2, MMP-7, MMP-9 and MMP-12, which also aid in neovascularization. Thus, highly vascularized tumors usually contain large numbers of TAMs[7,8]. Finally, TAMs promote metastasis through production of cytokines, such as TNFα, IL-1β and the release of MMPs to aid in the dissemination of cancer cells, which occurs most frequently close to TAMs and thus high TAM infiltration and presence in tumor tissue correlates frequently with a bad patient prognosis[9,10].

Interleukin-1β.

There is ample evidence for a tumor promoting role of local low level chronic inflammation and the infiltration of macrophages into the tumor microenvironment. One of the early on produced and highly potent pro-inflammatory cytokine is interleukin (IL)-1β, which is primarily produced by monocytes and macrophages. IL-1 was the first cytokine discovered. It mediates diverse biological functions and had been known by various identifiers, including endogenous pyrogen, pyrexin, catabolin, lymphocyte activating factor, leukocytic endogenous mediator, mononuclear cell factor, osteoclast activating factor or hemopoietin[11]. Subsequently it was shown that these responses were actually mediated by two related cytokines IL-1α and IL-1β, which have very related function and signal through the same receptor complex[12]. Two IL-1 receptors exist, IL-1RI and the decoy IL-1RII, which lacks most of the cytosolic signaling domain. IL-1 signaling is further regulated through soluble receptors and the requirement of the IL-1R accessory protein (IL-1RAc) for conversion into the high affinity, signaling competent receptor complex. The naturally occurring IL-1R antagonist (IL-1Ra) competes with IL-1 for receptor binding, but does not initiate signal transduction[13]. However, while IL-1α remains primarily intracellular, IL-1β is released from cells by an unconventional mechanism into the circulation. IL-1β is a potent pro-inflammatory cytokine and essential for the inflammatory host response to infection and tissue damage, it is now also firmly linked to the development of chronic inflammatory and immune diseases, including cancer.

Regulation of IL-1β production is unique among cytokines and only shared with the related IL-1 family cytokine IL-18[13]. Most inflammatory cytokines, including IL-1β, IL-6, IL-8 or TNFα, are regulated through inducible transcription. Accordingly, IL-1β is absent under normal conditions, but inflammation and stress cause rapid up regulation of its transcription, but also translation is uncoupled from transcription and regulated independently[14]. However, in contrast to most inflammatory cytokines, transcription and translation of IL-1β produces only an inactive precursor (pro-IL-1β), and the generation of the biologically active, mature IL-1β requires

additional proteolytical processing to remove its pro-domain[15]. Mature IL-1β is then released by an atypical, leader peptide-independent mechanism, which is still controversial[16]. Maturation of IL-1β is accomplished by the cysteine-aspartic protease caspase-1, which cleaves the precursor following Asp[116] to liberate the 17 kDa mature cytokine[17,18]. Also caspase-1 is initially produced as a zymogen, pro-caspase-1, and requires activation, which occurs in an inducible protein complex, termed inflammasome[19]. Caspase-1 together with caspases-4 and -5 (and their mouse paralogue caspase-11), and caspase-12 form the inflammatory caspase subfamily, which are initiator caspases and contain a caspase recruitment domain (CARD). The CARD is essential for the clustering of pro-caspases required for their autocatalytic transactivation by the induced proximity mechanism, and this close proximity occurs in the inflammasomes[19].

Inflammasome.

The inflammasome represents a host defense system, which is part of the innate immune system based on the recognition of infection and tissue damage by germ-line encoded cytosolic pattern recognition receptors (PRRs). The PRRs that activate inflammasomes belong to the Nod-like receptors (NLRs) and AIM2-like receptors (ALRs), though also the cytosolic RNA-sensor RIG-I activates inflammasomes[20]. NLRs display a tripartite domain architecture, consisting of a C-terminal leucine rich region (LRR), a central nucleotide binding NACHT domain, and an N-terminal effector domain crucial for downstream signaling, which in the majority of NLRs is a CARD (NLRCs) or a PYRIN domain (PYD) (NLRPs). Both are protein-protein interaction domains and required for assembly of inflammasomes. The LRR is the putative ligand recognition domain and responsible for NLR activation, and accordingly, in vivo deletion of the LRR of NLRP3 renders the protein unresponsive[21]. The NACHT contains a NTPase domain as demonstrated for NLRP1, NLRP3 and NLRP12[22-24]. Although 22 human NLR and 33 mouse Nlr genes exist, the function of most NLRs has yet to be elucidated and only a few NLRs have been linked to inflammasome activation. Little is known about the nature of most NLR ligands. NLRP1 is involved in the recognition of the peptidoglycan component muramyl-dipeptide (MDP) and the lethal toxin (LeTx) of *B. anthraces* in a complex with

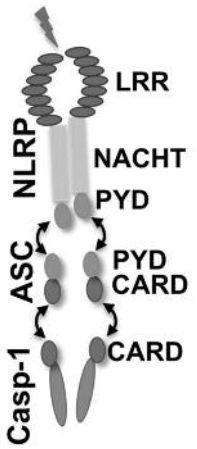

Figure 1: Formation of the inflammasome by NLRs.
Activated NLRs recruit the adaptor ASC by PYD-mediated interaction, which then brings pro-caspase-1 into the complex by CARD-mediated interaction.

Nod2[25]. NLRP2 assembles an inflammasome in vitro, but its ligand and in vivo relevance is still elusive. NLRP3, perhaps the best studied NLRP, senses a large number of agonists by multiple mechanisms that include potassium efflux, lysosomal damage and generation of reactive oxygen species, suggesting an indirect mechanism for agonist sensing[26]. NLRC4 is activated by intracellular bacteria via recognition of cytosolic flagellin[27]. In addition, NLRC4 recognizes the rod structure of bacterial type III secretion systems from several pathogens[28,29]. Increasing evidence supports that NLRs also heterodimerize. NLRP1/Nod2 complexes mediate MDP and LeTx recognition[25]. Similarly, flagellin is recognized by NLRC4 in concert with NLRB1[30]. As one can expect, there is increasing evidence that multiple NLRs synergistically recognize microbial infections, likely via distinct pathogen associated molecular patterns (PAMPs). For example, *Listeria monocytogenes* infection is recognized by NLRP3, NLRC4, and AIM2[31]. The ALR AIM2 is the PRR that directly recognizes cytosolic DNA to mediate the host response through activation of inflammasomes[32,33]. AIM2 belongs to the HIN-200 family of interferon-response genes and consists of an N-terminal PYD and a HIN-200 domain, and AIM2 recognizes cytosolic dsDNA, including synthetic DNA, viruses and bacterial DNA and genomic DNA. Infection of AIM2 deficient macrophages with the bacterial pathogens *Francisella tularensis* and *L. monocytogenes*, or the DNA viruses vaccinia virus, HSV-1 and CMV results in impaired caspase-1 activation, IL-1β release and

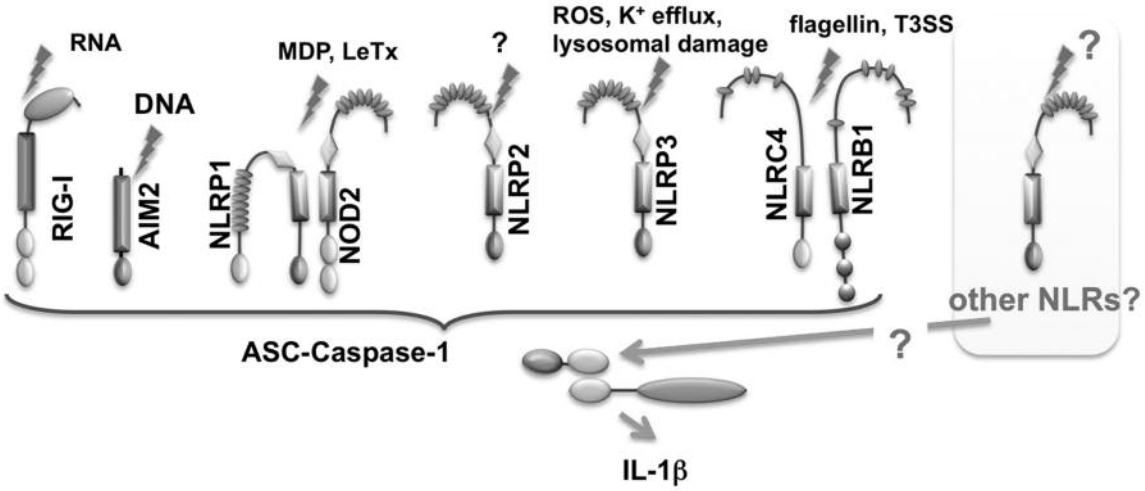

Figure 2: Activation of PRRs involved in inflammasome-mediated caspase-1 activation and IL-1β maturation and release.

pathogen clearance[32,33]. The PYD in NLRs and AIM2 is the protein interaction domain required for recruitment of the adaptor protein ASC, which in turn recruits pro-caspase-1 to form the caspase-1-activating inflammasome (Figure 1,2)[34,35].

Besides responding to PAMPs, inflammasomes also respond to tissue damage and sterile inflammation through released danger associated molecular patterns (DAMPs). NLRP3 is activated by exogenous ATP, uric acid crystals, Amyloid beta, Cholesterol crystals and hyaluronan, while AIM2 is activated by cytosolic DNA released by cell damage[36]. Accordingly, activation of inflammasomes have been linked to numerous inflammatory diseases, which are commonly characterized by excessive production of IL-1β, including periodic fever syndromes, gouty arthritis, systemic onset juvenile idiopathic arthritis, Still's disease, System lupus erythematosus, multiple sclerosis, asthma, Chron's disease, atherosclerosis, Alzheimer's disease or type 2 diabetes[37].

While macrophages require inflammasomes and thus caspase-1 for IL-1β release, several other cell types can release IL-1β, which often is pro-IL-1β, such as IL-1β released from short lived dying or damaged cells. For example, neutrophils and mast cells release serine proteases that are frequently present in inflammatory fluids. These proteases, including proteinase-3, elastase, chymase, matrix metalloproteinases and others can also mature pro-IL-1β[38].

Interleukin-1β and cancer.

Since IL-1β is a pleiotropic pro-inflammatory mediator and most cancers have an inflammatory component, IL-1β has been linked to tumorigenesis[39]. Although, IL-1β is primarily produced by TAMs, some cancer cells also acquired this capability, and thus its cancer promoting effects have been attributed to autocrine and paracrine-mediated up regulation of pro-angiogenic factors to promote neovascularization, such as VEGF, IL-8, TNFα, CXCL2, HGF and TGFβ and metastasis, including MMPs, adhesion molecules and growth factors (Figure 3)[14]. Several studies clearly demonstrated a correlation between high expression of IL-1β in cancer and a poor prognosis for patients, including head and neck cancer, colon cancer, lung cancer, melanoma, breast cancer, leukemia and myeloma[14,40-42]. *IL-1B* gene polymorphisms causing increased expression of IL-1β have been linked to an increased risk of gastric cancer following *H.*

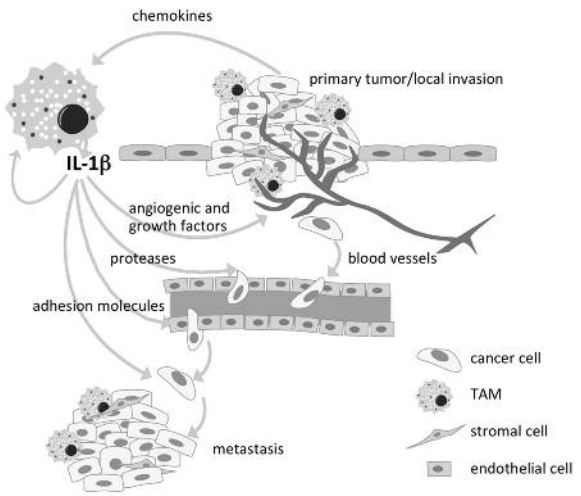

Figure 3: IL-1β-mediated effects that promote tumorigenesis.

pylori infection[43]. Similar results were obtained in numerous studies for different ethnic populations, but not for all populations analyzed. Further correlation was detected to cervical cancer, gallbladder cancer, breast cancer and esophageal cancer. Metastatic pancreatic cancer cell lines exhibit higher expression of the IL-RI than non-metastatic lines[44]. Ectopic expression of IL-1β in lung cancer cells did not show any changes in proliferation in vitro, but causes a significant increase in tumor growth in vivo and tumors showed significant increase in vascularization, supporting a paracrine mechanism[42]. Parietal cell-specific expression of IL-1β in transgenic mice caused spontaneous gastric inflammation and adenocarcinomas[45]. Support for the metastatic function of IL-1β is emphasized by a study showing that administering IL-1β before the i.v. injection of melanoma cells augmented lung metastasis[46]. Compelling evidence came finally from Voronov and colleagues, who demonstrated in xenograft tumor models that IL-1β is essential for tumor neovascularization, thus growth, and invasiveness using IL-1β deficient mice, thus IL-1β deficient mice showed no metastasis and survived, while wild type mice succumbed to lung metastasis, and co-cultured IL-1β deficient TAMs with melanoma cells released reduced levels of VEGF and TNFα[47]. Similar results were also obtained in models of metastatic breast and prostate cancer[47].

As discussed above, IL-1β signaling is regulated by the naturally occurring inhibitory protein IL-1Ra. Since in healthy individuals IL-1Ra is present in excess compared to IL-1β, the concept is that in inflammatory disease the local and circulating levels of IL-1Ra are insufficient to block IL-1β signaling. Thus, in cancer low levels of IL-1Ra might fail to prevent the angiogenic and metastatic properties of IL-1β. This scenario is observed in several forms of hematopoietic tumors, including lymphomas, multiple myeloma and leukemia, which show constitutively elevated levels of IL-1β and reduced levels of IL-1Ra, which however is not a unified model for all cancers,and elevated levels of IL-1Ra exist in tumors that are associated with more severe forms[14,48,49]. However, since IL-1Ra is expressed as a consequence of IL-1β signaling, it might merely indicate excessive production of IL-1β[39]. Based on the convincing studies that IL-1β promotes angiogenesis and metastasis, several studies investigated application of the recombinant IL-1Ra in tumor mouse models and demonstrated the efficacy of IL-1Ra for cancer therapy. First, similar to *IL-1B* gene polymorphisms, also polymorphisms in the *IL-1RN* gene, which encodes IL-1Ra, are linked to an increased risk for gastric, colorectal, cervical, gallbladder, esophageal, lung and prostate cancer[43]. IL-1Ra-deficient mice develop tumors following exposure to the chemical carcinogen 3-MCA, while only some IL-1β deficient mice developed tumors, which are smaller and lacked neutrophil and macrophage infiltration[50]. Fibrosarcoma cell lines derived from the 3-MCA primary tumors from IL-1Ra deficient mice were most aggressive and metastatic in an experimental metastasis model, while cell lines from IL-1β deficient mice showed the lowest number of lung metastases[50]. Along these lines, expression of IL-1Ra in skin carcinoma cells significantly reduced tumor growth in xenograft mouse models[51]. Melanoma xenograft growth and metastasis in nude mice was strongly reduced, when cells were transduced with IL-1Ra[52]. Those and similar studies strongly support a role of IL-1Ra in preventing tumorigenesis.

Melanoma xenograft growth is inhibited in vivo, when IL-1Ra is administered at the same time as melanoma cells and also improved efficacy of chemotherapy[53]. Administering IL-1Ra in mouse melanoma models reduced lung and liver metastases, which has been attributed to the impaired expression of adhesion molecules[54-56]. Yet another study demonstrated that administering IL-1Ra shows efficacy as either preventative or as a treatment regimen, which reduced the liver metastasis burden by 80% caused by intrasplenic injection of melanoma cells[56]. Voronov and colleagues treated wild type mice with IL-1Ra, which resulted in significantly reduced tumor vascularization in a melanoma mouse model[47]. A xenograft mouse study evaluating the primary tumor growth also demonstrated a beneficial effect from IL-1Ra treatment of melanoma, colon adenocarcinoma and lung adenocarcinoma cell lines that produce IL-1β, but not that of a melanoma and squamous cell carcinoma cell line lacking IL-1β expression[57]. As discussed above, the stomach-specific expression of IL-1β caused spontaneous gastric cancer, which could be prevented by a 6-week treatment regimen using IL-1Ra[45].

Several mouse studies also explored novel continuous delivery methods for IL-1Ra to treat fibrosarcoma with microencapsulated genetically engineered cells that secrete IL-1Ra, or melanoma xenograft growth with continuous release of IL-1Ra from biodegradable microspheres, providing evidence for the successful long-term application of IL-Ra[58,59]. Anakinra is a recombinant, non-glycosylated form of the IL-1Ra, which is a well established and tolerated approach to interfere with excessive circulating IL-1β in several autoinflammatory and autoimmune diseases, such as Rheumatoid Arthritis, Cryopyrinopathies, Familial Mediterranean fever, and others[60]. Due to some disadvantages, such as a short half life of 4 hours that require daily injections and injection site irritations, novel anti-IL-1β therapeutics have been recently developed, including humanized monoclonal anti-IL-1β antibody (Canakinumab), which only requires bi-monthly administration and which is approved for Cryopyrinopathies. Rilanocept (IL-1 Trap) is a fusion protein between the extracellular domains of the IL-1RAc and IL-1RI with the Fc portion of human IgG1, which allows high affinity interaction with, and sequestration of, IL-1β, and is also approved for the treatment of Cryopyrinopathies. Of note is that anakinra has been used for the long-term treatment of arthritis patients without adverse side effects, emphasizing the excellent safety profile of this drug[39].

Anti-IL-1β clinical trials.

Based on such promising results from mouse models, 2 clinical trials have been initiated to test the application of IL-1Ra in cancer patients. A phase I study from the National Cancer Institute (NCI) investigates anakinra for treating patients with metastatic cancer expressing the IL-1β gene (NCT00072111), which is still ongoing. A phase II study from the Mayo Clinic investigates patients with smoldering or indolent multiple myeloma (NCT00635154)[61]. Multiple myeloma, a B-cell malignancy has benign preconditions, including smoldering multiple myeloma (SMM) and indolent multiple myeloma (IMM). SMM patients have a median progression to active myeloma of 26 months and IMM patients of only 8-10 months. 47 SMM/IMM patients were enrolled and received anakinra (100 mg/d) for 6 months and non-responders and patients with stable disease received then in addition a low dose dexamethasone (20 mg/wk), which induces myeloma cell death in vitro when combined with IL-1β inhibition[61]. Initial results from this study indicate that anti-IL-1β therapy can significantly increase progression free survival (PFS) of high risk patients. The median PFS was 37.5 months and disease stability was achieved in 8 patients for 4 years while receiving therapy, which is still ongoing[61]. This first clinical trial provides a compelling argument for application of anti-IL-1β therapies in cancer patients to achieve the conversion into a manageable chronic disease, and thus warrants further studies in metastatic disease and highly vascularized tumors.

Our research focus.

Our laboratory studies the molecular mechanisms that control the maturation step of pro-IL-1β in macrophages and we are focusing on 2 aspects: 1) we investigate the signals that activate several novel NLRs and test their contribution to inflammasome activation. 2) we identified a family of inflammasome inhibitors, which we termed PYRIN domain-only proteins (POPs)[62]. POPs regulate the recruitment of the adaptor ASC to NLRs/ALRs in vitro and we are currently testing their function in vivo using novel macrophage-specific mouse models as well as targeting the inflammasome using recombinant POPs.

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References

1 Grivennikov, S.I., Greten, F.R. & Karin, M. Immunity, Inflammation, and Cancer. *Cell* 140, 883-899 (2010).

2 Aggarwal, B.B., Vijayalekshmi, R.V. & Sung, B. Targeting Inflammatory Pathways for Prevention and Therapy of Cancer: Short-Term Friend, Long-Term Foe. *Clinical Cancer Research* 15, 425-430 (2009).

3 Kuper, H., Adami, H.O. & Trichopoulos, D. Infections as a major preventable cause of human cancer. *Journal of Internal Medicine* 248, 171-183 (2000).

4 Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* 357, 539-545 (2001).

5 Mantovani, A., Sozzani, S., Locati, M., Allavena, P. & Sica, A. Macrophage polarization: tumor-associated

macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 23, 549-555 (2002).

6 Pollard, J.W. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 4, 71-78 (2004).

7 Leek, R.D., Lewis, C.E., Whitehouse, R., Greenall, M., Clarke, J. & Harris, A.L. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 56, 4625-4629 (1996).

8 Nishie, A., Ono, M., Shono, T., Fukushi, J., Otsubo, M., Onoue, H., Ito, Y., Inamura, T., Ikezaki, K., Fukui, M., Iwaki, T. & Kuwano, M. Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. *Clin Cancer Res* 5, 1107-1113 (1999).

9 Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2, 161-174 (2002).

10 Pollard, J.W. Macrophages define the invasive microenvironment in breast cancer. *J Leukoc Biol* 84, 623-630 (2008).

11 Dinarello, C.A. IL-1: discoveries, controversies and future directions. *Eur J Immunol* 40, 599-606 (2010).

12 Cameron, P., Limjuco, G., Rodkey, J., Bennett, C. & Schmidt, J.A. Amino acid sequence analysis of human interleukin 1 (IL-1). Evidence for biochemically distinct forms of IL-1. *J Exp Med* 162, 790-801 (1985).

13 Dinarello, C.A. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* 27, 519-550 (2009).

14 Dinarello, C.A. Biologic basis for interleukin-1 in disease. *Blood* 87, 2095-2147. (1996).

15 Black, R.A., Kronheim, S.R., Cantrell, M., Deeley, M.C., March, C.J., Prickett, K.S., Wignall, J., Conlon, P.J., Cosman, D., Hopp, T.P. & et al. Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor. *J Biol Chem* 263, 9437-9442 (1988).

16 Wewers, M.D. IL-1beta: an endosomal exit. *Proc Natl Acad Sci USA* 101, 10241-10242 (2004).

17 Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Salfeld, J., Towne, E., Tracey, D., Wardwell, S., Wei, F.-Y., Wong, W., Kamen, R. & Seshadri, T. Mice deficient in IL-1b-converting enzyme are defective in production of mature IL-1b and resistant to endotoxic shock. *Cell* 80, 401-411 (1995).

18 Kuida, K., Lippke, J.A., Ku, G., Harding, M.W., Livingston, D.J., Su, M.S. & Flavell, R.A. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science* 267, 2000-2003 (1995).

19 Martinon, F., Burns, K. & Tschopp, J. The Inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-1b. *Mol. Cell* 10, 417-426 (2002).

20 Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschlager, N., Schlee, M., Rothenfusser, S., Barchet, W., Kato, H., Akira, S., Inoue, S., Endres, S., Peschel, C., Hartmann, G., Hornung, V. & Ruland, J. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol* 11, 63-69 (2010).

21 Hoffman, H.M., Scott, P., Mueller, J.L., Misaghi, A., Stevens, S., Yancopoulos, G.D., Murphy, A., Valenzuela, D.M. & Liu-Bryan, R. Role of the leucine-rich repeat domain of cryopyrin/NALP3 in monosodium urate crystal-induced inflammation in mice. *Arthritis Rheum* 62, 2170-2179 (2010).

22 Ye, Z., Lich, J.D., Moore, C.B., Duncan, J.A., Williams, K.L. & Ting, J.P. ATP binding by monarch-1/NLRP12 is critical for its inhibitory function. *Mol Cell Biol* 28, 1841-1850 (2008).

23 Faustin, B., Lartigue, L., Bruey, J.M., Luciano, F., Sergienko, E., Bailly-Maitre, B., Volkmann, N., Hanein, D., Rouiller, I. & Reed, J.C. Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol Cell* 25, 713-724 (2007).

24 Duncan, J.A., Bergstralh, D.T., Wang, Y., Willingham, S.B., Ye, Z., Zimmermann, A.G. & Ting, J.P. Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. *Proc Natl Acad Sci USA* 104, 8041-8046 (2007).

25 Hsu, L.C., Ali, S.R., McGillivray, S., Tseng, P.H., Mariathasan, S., Humke, E.W., Eckmann, L., Powell, J.J., Nizet, V., Dixit, V.M. & Karin, M. A NOD2-NALP1 complex mediates caspase-1-dependent IL-1beta secretion in response to Bacillus anthracis infection and muramyl dipeptide. *Proc Natl Acad Sci USA* 105, 7803-7808 (2008).

26 Tschopp, J. & Schroder, K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nature Reviews Immunology* 10, 210-215 (2010).

27 Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T.D., Ozoren, N., Jagirdar, R., Inohara, N., Vandenabeele, P., Bertin, J., Coyle, A., Grant, E.P. & Nunez, G. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat. Immunol.* 7, 576-582 (2006).

28 Miao, E.A., Alpuche-Aranda, C.M., Dors, M., Clark, A.E., Bader, M.W., Miller, S.I. & Aderem, A. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat Immunol.* 7, 569-575 (2006).

29 Miao, E.A., Mao, D.P., Yudkovsky, N., Bonneau, R., Lorang, C.G., Warren, S.E., Leaf, I.A. & Aderem, A. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci USA* 107, 3076-3080 (2010).

30 Zamboni, D.S., Kobayashi, K.S., Kohlsdorf, T., Ogura, Y., Long, E.M., Vance, R.E., Kuida, K., Mariathasan, S., Dixit, V.M., Flavell, R.A., Dietrich, W.F. & Roy, C.R. The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of Legionella pneumophila infection. *Nat Immunol* 7, 318-325 (2006).

31 Wu, J., Fernandes-Alnemri, T. & Alnemri, E.S. Involvement of the AIM2, NLRC4, and NLRP3 Inflammasomes in Caspase-1 Activation by Listeria monocytogenes. *J Clin Immunol* (2010).

32 Fernandes-Alnemri, T., Yu, J.W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., McCormick, M., Huang, L., McDermott, E., Eisenlohr, L., Landel, C.P. & Alnemri, E.S. The AIM2 inflammasome is critical for innate immunity to Francisella tularensis. *Nat Immunol* 11, 385-393 (2010).

33 Rathinam, V.A., Jiang, Z., Waggoner, S.N., Sharma, S., Cole, L.E., Waggoner, L., Vanaja, S.K., Monks, B.G., Ganesan, S., Latz, E., Hornung, V., Vogel, S.N., Szomolanyi-Tsuda, E. & Fitzgerald, K.A. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat Immunol* 11, 395-402 (2010).

34 Srinivasula, S.M., Poyet, J.-L., Razmara, M., Datta, P., Zhang, Z. & Alnemri, E.S. The PYRIN-CARD protein ASC is an activating adaptor for Caspase-1. *J. Biol. Chem.* 277, 21119-21122 (2002).

35 Stehlik, C., Lee, S.H., Dorfleutner, A., Stassinopoulos, A., Sagara, J. & Reed, J.C. Apoptosis-associated speck-like protein containing a caspase recruitment domain is a regulator of procaspase-1 activation. *J. Immunol.* 171, 6154-6163 (2003).

36 Martinon, F., Mayor, A. & Tschopp, J. The inflammasomes: guardians of the body. *Annu Rev Immunol* 27, 229-265 (2009).

37 Sims, J.E. & Smith, D.E. The IL-1 family: regulators of immunity. *Nat Rev Immunol* 10, 89-102 (2010).

38 Stehlik, C. Multiple interleukin-1beta-converting enzymes contribute to inflammatory arthritis. *Arthritis Rheum* 60, 3524-3530 (2009).

39 Dinarello, C.A. Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev* 29, 317-329 (2010).

40 Gemma, A., Takenaka, K., Hosoya, Y., Matuda, K., Seike, M., Kurimoto, F., Ono, Y., Uematsu, K., Takeda, Y., Hibino, S., Yoshimura, A., Shibuya, M. & Kudoh, S. Altered expression of several genes in highly metastatic subpopulations of a human pulmonary adenocarcinoma cell line. *Eur J Cancer* 37, 1554-1561 (2001).

41 Chen, Z., Malhotra, P.S., Thomas, G.R., Ondrey, F.G., Duffey, D.C., Smith, C.W., Enamorado, I., Yeh, N.T., Kroog, G.S., Rudy, S., McCullagh, L., Mousa, S., Quezado, M., Herscher, L.L. & Van Waes, C. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clin Cancer Res* 5, 1369-1379 (1999).

42 Saijo, Y., Tanaka, M., Miki, M., Usui, K., Suzuki, T., Maemondo, M., Hong, X., Tazawa, R., Kikuchi, T., Matsushima, K. & Nukiwa, T. Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: in vivo analysis of tumor-stromal interaction. *J Immunol* 169, 469-475 (2002).

43 El-Omar, E.M., Carrington, M., Chow, W.H., McColl, K.E., Bream, J.H., Young, H.A., Herrera, J., Lissowska, J., Yuan, C.C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J.F., Jr. & Rabkin, C.S. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 404, 398-402 (2000).

44 Sawai, H., Funahashi, H., Yamamoto, M., Okada, Y., Hayakawa, T., Tanaka, M., Takeyama, H. & Manabe, T. Interleukin-1alpha enhances integrin alpha(6)beta(1) expression and metastatic capability of human pancreatic cancer. *Oncology* 65, 167-173 (2003).

45 Tu, S., Bhagat, G., Cui, G., Takaishi, S., Kurt-Jones, E.A., Rickman, B., Betz, K.S., Penz-Oesterreicher, M., Bjorkdahl, O., Fox, J.G. & Wang, T.C. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 14, 408-419 (2008).

46 Giavazzi, R., Garofalo, A., Bani, M.R., Abbate, M., Ghezzi, P., Boraschi, D., Mantovani, A. & Dejana, E. Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res* 50, 4771-4775 (1990).

47 Voronov, E., Shouval, D.S., Krelin, Y., Cagnano, E., Benharroch, D., Iwakura, Y., Dinarello, C.A. & Apte, R.N. in *Proc Natl Acad Sci USA*, Vol. 100 2645-2650 (2003).

48 Rambaldi, A., Torcia, M., Bettoni, S., Vannier, E., Barbui, T., Shaw, A.R., Dinarello, C.A. & Cozzolino, F. Modulation of cell proliferation and cytokine production in acute myeloblastic leukemia by interleukin-1 receptor antagonist and lack of its expression by leukemic cells. *Blood* 78, 3248-3253 (1991).

49 Estrov, Z., Kurzrock, R., Estey, E., Wetzler, M., Ferrajoli, A., Harris, D., Blake, M., Gutterman, J.U. & Talpaz, M. Inhibition of acute myelogenous leukemia blast proliferation by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors. *Blood* 79, 1938-1945 (1992).

50 Krelin, Y., Voronov, E., Dotan, S., Elkabets, M., Reich, E., Fogel, M., Huszar, M., Iwakura, Y., Segal, S., Dinarello, C.A. & Apte, R.N. Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res* 67, 1062-1071 (2007).

51 La, E., Rundhaug, J.E. & Fischer, S.M. Role of intracellular interleukin-1 receptor antagonist in skin carcinogenesis. *Mol Carcinog* 30, 218-223 (2001).

52 Weinreich, D.M., Elaraj, D.M., Puhlmann, M., Hewitt, S.M., Carroll, N.M., Feldman, E.D., Turner, E.M., Spiess, P.J. & Alexander, H.R. Effect of interleukin 1 receptor antagonist gene transduction on human melanoma xenografts in nude mice. *Cancer Res* 63, 5957-5961 (2003).

53 Triozzi, P.L. & Aldrich, W. Effects of interleukin-1 receptor antagonist and chemotherapy on host-tumor interactions in established melanoma. *Anticancer Res* 30, 345-354 (2010).

54 Chirivi, R.G., Garofalo, A., Padura, I.M., Mantovani, A. & Giavazzi, R. Interleukin 1 receptor antagonist inhibits the augmentation of metastasis induced by interleukin 1 or lipopolysaccharide in a human melanoma/nude mouse system. *Cancer Res* 53, 5051-5054 (1993).

55 Vidal-Vanaclocha, F., Fantuzzi, G., Mendoza, L., Fuentes, A.M., Anasagasti, M.J., Martin, J., Carrascal,

T., Walsh, P., Reznikov, L.L., Kim, S.H., Novick, D., Rubinstein, M. & Dinarello, C.A. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci USA* 97, 734-739 (2000).

56 Vidal-Vanaclocha, F., Amezaga, C., Asumendi, A., Kaplanski, G. & Dinarello, C.A. Interleukin-1 receptor blockade reduces the number and size of murine B16 melanoma hepatic metastases. *Cancer Res* 54, 2667-2672 (1994).

57 Elaraj, D.M., Weinreich, D.M., Varghese, S., Puhlmann, M., Hewitt, S.M., Carroll, N.M., Feldman, E.D., Turner, E.M. & Alexander, H.R. The role of interleukin 1 in growth and metastasis of human cancer xenografts. *Clin Cancer Res* 12, 1088-1096 (2006).

58 Lavi, G., Voronov, E., Dinarello, C.A., Apte, R.N. & Cohen, S. Sustained delivery of IL-1 Ra from biodegradable microspheres reduces the number of murine B16 melanoma lung metastases. *J Control Release* 123, 123-130 (2007).

59 Bar, D., Apte, R.N., Voronov, E., Dinarello, C.A. & Cohen, S. A continuous delivery system of IL-1 receptor antagonist reduces angiogenesis and inhibits tumor development. *FASEB J* 18, 161-163 (2004).

60 Dinarello, C.A. Blocking IL-1 in systemic inflammation. *J. Exp. Med.* 201, 1355-1359 (2005).

61 Lust, J.A., Lacy, M.Q., Zeldenrust, S.R., Dispenzieri, A., Gertz, M.A., Witzig, T.E., Kumar, S., Hayman, S.R., Russell, S.J., Buadi, F.K., Geyer, S.M., Campbell, M.E., Kyle, R.A., Rajkumar, S.V., Greipp, P.R., Kline, M.P., Xiong, Y., Moon-Tasson, L.L. & Donovan, K.A. Induction of a chronic disease state in patients with smoldering or indolent multiple myeloma by targeting interleukin 1{beta}-induced interleukin 6 production and the myeloma proliferative component. *Mayo Clin Proc* 84, 114-122 (2009).

62 Stehlik, C. & Dorfleutner, A. COPs and POPs: Modulators of Inflammasome Activity. *J Immunol* 179, 7993-7998 (2007).