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Mini-Review

# Redox control of NLRP3 inflammasome activation in health and disease

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## ABSTRACT

**Shifts in the redox balance between ROS and antioxidants regulate innate immunity at various levels. Changes in the redox microenvironment modulate the activation potential of the NLRP3 inflammasome, a signaling platform that activates caspase-1, allowing the maturation of IL-1β. However, a clear definition of the underlying mechanism is missing. In this essay, I review the most-credited theories on inflammasome activation. In particular, I will focus on the redox-mediated mechanisms that regulate the assembly of NLRP3 inflammasome and discuss how aberrations in them are implicated in the pathogenesis of autoinflammatory diseases. *J. Leukoc. Biol.* 92: 951–958; 2012.**

## Introduction

IL-1 is one of the most powerful inflammatory mediators. It is responsible for acute attacks of systemic or local inflammation and also contributes to several chronic diseases. Increasing evidence indicates that slowly progressive inflammatory processes linked to dysregulated production of IL-1 are implicated in the pathophysiology of several common diseases, including atherosclerosis, osteoarthritis, metabolic syndrome, and type 2 diabetes, of the Western countries [1].

The story of IL-1 begins in the early 1940s with investigations into the nature of the endogenous protein produced by leukocytes that causes fever. These studies eventually led to the cloning of IL-1α [2] and IL-1β [3]. Surprisingly, the cDNA analyses revealed two features unusual for cytokines: first, the two proteins are produced as precursor polypeptides; second, and most unexpected, the amino terminus of IL-1α and -β does not possess the hydrophobic signal (or leader sequence) common to most secreted (and membrane) proteins, which allows them to enter the exocytotic pathway [4].

The finding that IL-1α and -β, in spite of their extracellular localization and function, lack the secretory signal sequence raised the question of how can a leaderless protein leave the producing cell and reach the extracellular space [5]. The first and simplest explanation for this peculiarity was that cell death at the site of inflammation would result in release of the cytokine. This hypothesis turned out to be true for IL-1α but not IL-1β.

Although the two cytokines share the same receptor on target cells and induce the same proinflammatory effects, they display key differences. IL-1α and -β precursors accumulate in the cell cytosol and therefore, become available extracellularly when dying cells passively release their intracellular contents. However, whereas IL-1α is bioactive as an unprocessed precursor, the IL-1β precursor is not and requires proteolytic processing to become active. Moreover, IL-1α is present intracellularly in healthy tissues [6], whereas the expression of IL-1β is restricted to a few cell types upon activation [7]. Therefore, whereas IL-1α exploits cell death to exert its proinflammatory activities extracellularly, the same does not apply to IL-1β. Rather, IL-1β secretion is selective and occurs through a pathway alternative to the ER-Golgi one; living cells are required for secretion of the mature, fully processed cytokine [8]. Since the discovery of a novel pathway of secretion for IL-1β, several secretory proteins lacking signal peptides were identified, and the existence of a "leaderless" mechanism of secretion has been firmly established [5, 9].

As discussed by Charles Dinarello [7], IL-1α and -β are paradigmatic for cytokine evolution. First, cytokines were intracellular growth factors and repair molecules, interacting with DNA as transcription factors but not secreted proteins. Later, when Igs evolved, they were used by cytokine growth factors as cell surface receptors; thus, externalized cytokines found a second life. Some of them, such as IL-1α, retain intracellular (nuclear) function [6] but when released from injured cells, profit from the receptor to signal danger and are the archetypes of damage-associated molecular pattern molecules [10]. Others, including IL-1β and other IL-1 family members, such as IL-18, which evolved later, found their way to leave cells in a regulated manner.

Abbreviations: CAPS=cryopyrin-associated periodic syndromes, ICE=IL-1β-converting enzyme, IL-1Ra=IL-1R antagonist, LRR=leucine-rich repeat, NLR=nucleotide-binding domain and leucine-rich repeat containing, NLRP3= nucleotide-binding domain, leucine-rich-repeat-containing family, pyrin domain-containing 3, Nrf2=NF-erythroid-derived 2-related factor 2, TXNIP=thioredoxin inhibitory protein

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## PROCESSING OF IL-1β: FROM ICE TO THE INFLAMMASOME

IL-1β activity is controlled at several levels. First, the IL-1β gene is not constitutively expressed: triggering of TLR on inflammatory cells is required to drive IL-1β expression. Secondly, IL-1β mRNA is translated into a precursor protein, devoid of biological activity, which accumulates in the soluble cytosol. The inactive pro-IL-1β is then cleaved by caspase-1 into an active cytokine and secreted. The ICE (caspase-1) was isolated in 1992 [11, 12] and soon identified as a member of the caspase family [13]. ICE/caspase-1 is synthesized as a zymogen. The mechanism involved in its activation remained elusive until 2002, when Jurg Tschopp and his group [14] discovered a molecular platform triggering activation of caspase-1 and processing of pro-IL-1β (and pro-IL-18) and called it the inflammasome.

Different types of inflammasomes exist, each composed by a member of the NLR gene family, adaptor proteins, and procaspase-1 molecules. In the human system, 23 NLR genes have been identified so far, but only a few are inflammasome initiators [15]. Among these, the NLRP1 inflammasome assembles in response to muramyl dipeptide and anthrax lethal toxin, the NLRC4 inflammasome senses flagellin, and the NLRP3 inflammasome is engaged by various diverse stimuli, including pore-forming toxins, bacteria, viruses, and endogenous molecules. Recently, the protein absent in melanoma 2, which does not belong to the group of the NLR family, was proposed to form an inflammasome in response to cytosolic DNA [16, 17]. In this review, I will focus on NLRP3, the most important inflammasome in sterile inflammation.

## HOW IS NLRP3 INFLAMMASOME ACTIVATED?

The molecular components of the inflammasomes have been identified and thoroughly characterized, and the key role of the assembled complex in the maturation of IL-1β is widely recognized. However, where and how the various inflammasome components are assembled to form an active complex remains largely obscure [18].

Two steps may be distinguished in IL-1β secretion. In a first step, TLR agonists induce expression and synthesis of pro-IL-1β, which accumulates intracellularly; in a second step, exposure to a range of physically and chemically diverse substances of microbial, environmental, or endogenous origin, collectively named second signals, triggers inflammasome activation. The assembly of inflammasome brings procaspase-1 molecules close enough to induce their autoprocessing into active fragments. Caspase-1 then cleaves inactive IL-1β precursors to an active form, followed by externalization of the mature cytokine. Substances able to activate the inflammasomes (the second signals) include exogenous ATP, toxins, crystals, such as uric acid or cholesterol crystals, or fibers, such as asbestos and amyloid-β fibrils [15, 18].

Like the other NLRs, NLRP3 displays a tripartite structural organization consisting of C-terminal LRRs, a central nucleotide binding and oligomerization domain, and an N-terminal effector domain [15]. LRR domains are found in many proteins with a wide variety of biological functions. Although different in different proteins, these domains share a common motif of 20–30 aa rich in leucine, which makes them suitable for protein–protein interactions and ligand binding. For instance, TLRs sense PAMP molecules via their LRR domain.

In analogy to TLRs, LRRs in NLRs were believed to be potential ligand-binding domains that directly detect second signals. Nevertheless, a clear receptor–ligand interaction has not been demonstrated for any NLR yet. Rather, pathways generated downstream of second signals have been identified as possible mediators of NLRP3 inflammasome assembly (**Fig. 1**). The first identified was $K^+$ efflux [19], which was known to induce IL-1β processing and secretion long before the discovery of inflammasome [20, 21]. Many second signals, including pore-forming bacterial cytotoxins and extracellular ATP, which engages the ATP-gated cation channel P2X7 [22], induce the exit of $K^+$ from cells. However, the mechanism linking $K^+$ efflux (and the consequent intracellular depletion of $K^+$) to inflammasome oligomerization remains to be clarified (Fig. 1). A second mechanism indicates that phagolysosomal destabilization after the digestion of large crystalline particles causes the release into the cytosol of cathepsin proteases required for inflammasome activation [23]. This model implies that a cathepsin-cleavable substrate is a critical component for NLRP3 acti-

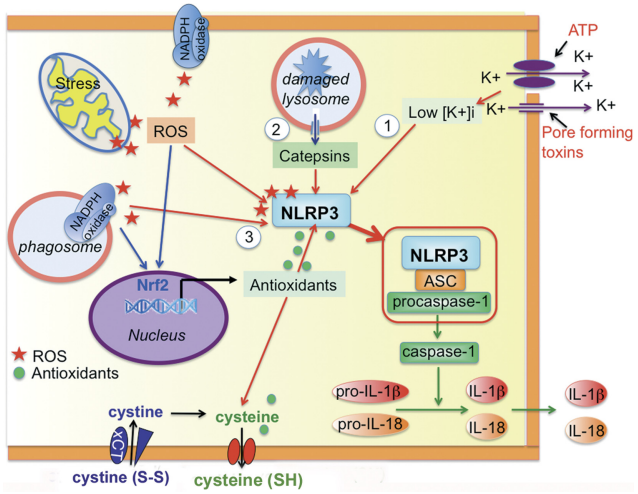


**Figure 1. Proposed mechanisms of NLRP3 inflammasome activation.** 1. Second signals, such as pore-forming toxins and exogenous ATP, induce $K^+$ efflux. The resulting low intracellular $K^+$ concentration ($[K^+]i$) favors inflammasome oligomerization. 2. Ingestion of second signals, such as crystals or fibers, may lead to lysosomal rupture with cytosolic leakage of cathepsins. These enzymes have been proposed to mediate inflammasome activation by cleaving NLRP3 or an inhibitor or activator thereof. 3. ROS, produced by stressed mitochondria or following activation of membrane NADPH oxidase, and antioxidants, up-regulated in response to ROS induction, have been reported to activate the inflammasome. Although the involvement of all of these mechanisms in inflammasome activation is supported by solid data, a mechanistic insight is still missing. ASC, Apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; xCT, cystine/glutamate exchanger; cystine (S-S), oxidized form of cysteine (two cysteine residues linked by a disulfide bond); cysteine (SH), reduced form of cysteine (containing a free thiol).

vation; however, so far, no such cathepsin substrate has been identified (Fig. 1).

## REDOX SIGNALING AND NLRP3 INFLAMMASOME ACTIVATION

The third mechanism proposed to mediate NLRP3 inflammasome activation involves the redox signaling (Fig. 1). The redox hypothesis arose from the in vitro evidence that exposure of myeloid cells to inflammasome-activating second signals, such as ATP or fibers, induces activation of NADPH oxidase with production of ROS [24–26]. In vitro inhibition of ROS generation by inhibitory compounds or knockdown of NADPH oxidase subunits revealed that in the absence of ROS production, caspase-1 activation and IL-1$\beta$ secretion are impaired, supporting the crucial role of NADPH oxidase-dependent ROS in inflammasome activation.

However, this conclusion has been challenged by several observations. Bauernfeind et al. [27] reported that NLRP3, like IL-1$\beta$, strictly requires priming by a proinflammatory signal (a step that is blocked by ROS inhibitors) and proposed that ROS production is necessary for NLRP3 induction but not activation. Furthermore, genetic data obtained in mice and humans argued against the strict requirement of ROS. In fact, IL-1$\beta$ processing and secretion triggered by various second signals are unaffected in macrophages from mice lacking NADPH oxidase activity [23, 28]. Likewise, in patients affected by chronic granulomatous disease, the impaired ROS production, as a result of a genetic defect of NADPH oxidase, does not inhibit—or even increases—IL-1$\beta$ secretion [29–31]. Later, the role of ROS was revisited, and ROS, generated by stressed mitochondria after exposure to second signals (rather than NADPH oxidase-derived ROS), were proposed to mediate inflammasome assembly [32]. More recently, the requirement of mitochondrial ROS has been reaffirmed by data showing a link among induction of apoptosis, ROS production, and NLRP3 inflammasome activation [33, 34]. Interestingly, Shimada et al. [34] propose a unifying theory of the three potential NLRP3 activating mechanisms, where $K^+$ efflux, lysosomal damage, and ROS converge to induce release of oxidized mitochondrial DNA into the cytosol, where it binds to and activates the NLRP3 inflammasome.

Quite unexpectedly, a mechanism of inflammasome activation mediated by antioxidants instead of ROS was also suggested [28]. In SOD1-deficient mice, the constitutively higher ROS levels in macrophages were shown to inhibit procaspase-1 activation by reversible oxidation of cysteine residues on the zymogen. The ROS-dependent inhibition of caspase-1 could be reversed by reducing agents that increase the cellular redox potential, convert the oxidized cystine residues to the reduced state, and allow caspase-1 activation.

In support of a direct involvement of antioxidants, recent data have indicated that the oxidative stress-responsive transcription factor Nrf2, which induces key antioxidant enzymes, is essential for cholesterol crystal-induced inflammasome activation [35] (Fig. 1).

A further discrepancy concerns the role of TXNIP. This protein was identified previously as a stress-responsive protein that binds to and inhibits the reducing enzyme thioredoxin, hence, a pro-oxidant factor [36]. More recently, TXNIP was found induced by high glucose and involved in insulin resistance [37]. Interestingly, high levels of glucose [38] and TXNIP [39] trigger IL-1$\beta$ expression. Glucose-induced IL-1$\beta$ is a key mediator of islet dysfunction and destruction, and the pathogenetic role of IL-1$\beta$ in type 2 diabetes is increasingly recognized [1]. On these bases, two groups independently investigated the possible role of TXNIP in inflammasome activation. Their studies provided opposite results. Zhou et al. [40] demonstrated the requirement of TXNIP during high glucose-mediated caspase-1 activation in murine pancreatic B cells. In particular, the authors proposed that ROS, induced by second signals, mediate the detachment of TXNIP from thioredoxin and its binding to NLRP3. Interaction with NLRP3 would promote inflammasome activation. In contrast, Masters et al. [41] reported no difference in caspase-1 activation and IL-1$\beta$ secretion in response to inflammasome activators (including islet amyloid polipeptide) between WT and TXNIP-deficient macrophages, thereby ruling out a role for TXNIP in regulating IL-1$\beta$ production by the inflammasome.

## WHY SO MANY CONTRASTING RESULTS?

A possible explanation of the discrepant observations reported on the role of pro-oxidant versus antioxidant events in inflammasome activation lies on the nature of the redox system itself, which works as a balance (**Fig. 2**). Every time an oxidative hit is provided to cells, and cells increase free radical production, ROS are detoxified rapidly by the redundant endogenous antioxidant systems—enzymatic and nonenzymatic—already present in cells [42]. When ROS production increases, up-regulation of several antioxidants occurs to restore the redox homeostasis. Small shifts within the homeostatic range mediate several physiological responses; more ample shifts are required for physiopathological processes such as inflammation. Therefore, the redox balance swings physiologically, and the transient (acute) oscillations in the redox state and the maintenance of a (chronic) equilibrium are fundamental for the correct cell functioning; when the equilibrium is lost, oxidative stress occurs [43, 44] (Fig. 2). This swinging property implies that in a cell system, it can be complicated to clearly dissect the role of ROS and antioxidants. For instance, in a given cell, a rise in pro-oxidants can be a result of increased generation of ROS or of a drop in the antioxidant capacity. The pharmacologic block of ROS production results in also blocking the antioxidant response [44, 45], making it difficult to understand whether ROS or the antioxidants, or maybe both, are involved in a given process, including the activation of the inflammasome. In addition, no standard unit for measuring "redox state" or "oxidant activity" at the cell level is available; only comparative measurements are possible. These and other limitations to the study of redox response pose considerable challenges for investigators in the field [43].

Most of the studies about inflammasome activation described above have investigated the redox remodelling that occurs after addition of second signals, which provide a strong, possibly nonphysiologic stress to cells. We have observed previ-

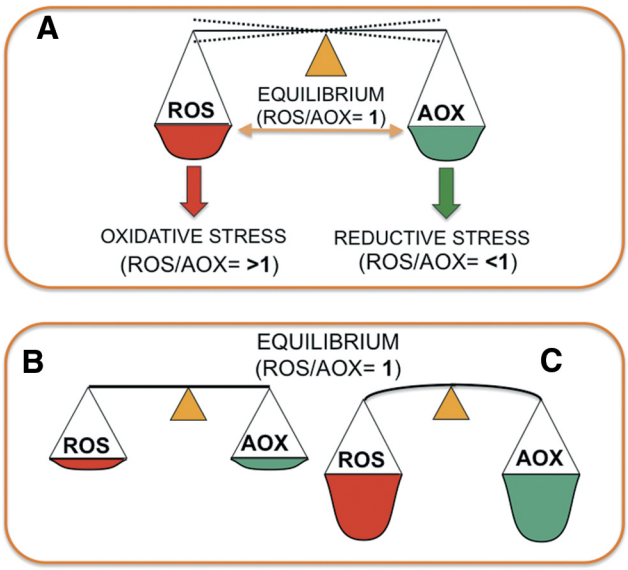


**Figure 2. The redox balance and its oscillations.** (A) Redox equilibrium is maintained by the balance of ROS and antioxidants (AOX; ROS/AOX=1). Physiologic oscillations are admitted (dotted lines). If ROS prevail, cells undergo oxidative stress. If antioxidants prevail, reductive stress may occur. (B and C) The redox equilibrium may be maintained with low (B) or high (C) levels of ROS and antioxidants in balance. However, a low level of antioxidants (B) allows cells to sustain strong oxidative hits, whereas when antioxidants are overexpressed (C), the redox balance is precarious, as a further increase of ROS may lead to the collapse of the antioxidant systems already expressed at a high level.

ously that in primary human monocytes, second signals enhance and accelerate processing and secretion of IL-1$\beta$ but are dispensable, as TLR agonists trigger secretion of endogenous ATP, which then activates autocrinally P2X7 receptors, resulting in inflammasome activation and IL-1$\beta$ processing and secretion, less strong than after addition of second signals but abundant and sustained in time [46]. In this more physiologic or at least less-stressed system, a biphasic redox response is induced by TLR triggering, with a first oxidative hit, resulting in a peak of ROS, followed by an antioxidant response [45]. The major antioxidant system up-regulated by ROS in LPS-activated monocytes is the cystine-cysteine redox cycle that mediates the uptake of oxidized cystine from the outside and its release in the reduced, free form, resulting in generation of an antioxidant state intra- and extracellularly (Fig. 1). This antioxidant mechanism, although less studied than others, is very important in the regulation of innate and adaptive immunity [47–49]. In PAMP-activated monocytes, the cystine-cysteine cycle is strongly up-regulated, and cysteine is externalized at high levels, with kinetics identical to that of IL-1$\beta$ secretion [45, 50]. The pro-oxidant and the antioxidant steps are required for IL-1$\beta$ processing, as blocking ROS production or the antioxidant response results in block of IL-1$\beta$ secretion without affecting the pro-IL-1$\beta$ intracellular accumulation [45].

Although these results reconciled in part the opposite views of ROS and antioxidants as main players in the process of inflammasome assembly, some controversies still remained. In particular, in some studies, the simple addition of antioxidant substances to the cell cultures inhibited IL-1$\beta$ secretion [25], whereas in other studies, the same antioxidants increased secretion [28, 45]. Likewise, down-modulation of thioredoxin turned out to promote [25] or inhibit [45] IL-1$\beta$ processing and secretion.

## THE BASAL REDOX STATE OF MYELOID CELLS DICTATES THEIR RESPONSE TO PRO-OXIDANT TRIGGERS AND THE RATE OF INFLAMMASOME ACTIVATION

In the studies mentioned above, the experimental setting was very similar, but different myeloid cells (i.e., human monocytes, continuous cell lines such as THP-1 cells, mouse macrophages from bone marrow, or peritoneum) were used. We then hypothesized that the basal redox state may differ in the different myeloid cells and be responsible for some of the reported discrepancies.

In general, adaptation of primary cells to continuous growth in vitro results in redox modifications as a result of several factors. First of all, cells are chronically exposed to high $O_2$ tension, which is nonphysiologic and pro-oxidant and induces a sustained overexpression of antioxidant genes. As oxido-reductases, such as thioredoxin, provide an advantage to growth, single cells overexpressing this enzyme are favored and selected during long-term culture [51]. Moreover, the cystine/cysteine cycle is up-regulated in many cell lines with accumulation of free thiols in the culture medium [52]. Therefore, continuous cell lines generally exhibit a more antioxidant phenotype than primary cells, which ensures faster growth and resistance to apoptosis but may cause aberrant redox remodeling to stimuli [53]. For instance, some macrophage cell lines, commonly used in inflammatory research, differ in their redox responses to external stimuli and consequently, in cytokine production [54].

Comparison of the basal redox state of primary monocytes and THP-1 cells confirmed that THP-1 cells have a higher reducing potential than freshly drawn primary monocytes with up-regulation of antioxidant systems [55]. Less expected, a strong prevalence of antioxidants is also found in short-cultured monocytes (48 h with GM-CSF) [55]. Unlike in fresh monocytes, TLR-mediated ROS induction was weak or absent in THP-1 cells and cultured monocytes and generated a weak antioxidant response. Remarkably, the rate of IL-1$\beta$ secretion was decreased dramatically compared with monocytes [55].

A likely interpretation is that in healthy monocytes, the redox homeostasis, maintained by a low production of ROS and a low expression of antioxidant systems, allows a strong redox response to TLR agonists with generation of the redox environment required for inflammasome assembly. Differently, in THP1 cells and macrophages, the oxidative hit provided by PAMPs is not strong enough to overcome the buffering capacity of the abundant antioxidants and results in low ROS generation. In turn, ROS at low level are not able to induce an antioxidant response. As a consequence, the weak redox remodeling induced by PAMPs is not strong enough to activate the

inflammasome efficiently, resulting in a low rate of IL-1β processing and secretion [56].

In support of this analysis, IL-1β processing and secretion by PAMP-stimulated THP-1 cells could be restored in two ways that both decrease the redox potential by 1) providing a stronger oxidative hit (i.e., 2 mM $H_2O_2$) [55] and 2) down-modulating antioxidant genes ([25] and unpublished results). In both cases, the biphasic redox response to TLR agonists is reestablished. The same manipulations in primary monocytes, which do not exhibit excess of antioxidants, result in a drop of IL-1β secretion and oxidative stress [45, 55].

The different redox state at baseline also explains the different sensitivity to reducing agents exhibited by primary monocytes and THP-1 cells [55]. In the presence of exogenous reductants, the rate of PAMP-induced IL-1β secretion increases in monocytes but decreases in THP-1 cells. Conceivably, a reducing environment is well-tolerated by monocytes, thanks to their balanced redox state with low antioxidant expression, and favors inflammasome activation. In contrast, THP-1 cells, as a result of their dramatically expanded antioxidant apparatus, are unable to further resist exposure to reducing agents and undergo a "reductive stress" [56]. Likewise, the opposite effects of thioredoxin down-modulation and reducing agents on inflammasome activation in THP-1 cells [25] and in primary monocytes [45, 55] are explained by the large excess of antioxidants in THP-1 cells compared with monocytes.

Therefore, the basal redox state of myeloid cells dictates amount, velocity, and efficiency of inflammasome activation in response to TLR agonists, resulting in different rates of bioactive IL-1β secretion [56].

## REDOX ALTERATION IN AUTOINFLAMMATORY DISEASES

The above data suggest caution about in vitro investigation on redox-related events carried out on tumor cell lines. However, it is possible that in vivo, under physiologic conditions, different redox microenvironments determine a higher or lower efficiency of inflammasome activation. Along this line, redox alterations, as found in some chronic diseases, may perturb the innate immune response by altering inflammasome activation and IL-1β secretion. This possibility was addressed by studying the redox state of monocytes from patients affected by CAPS. These are autoinflammatory disorders linked to mutations of *NLRP3* and characterized by severe inflammatory symptoms [57]. The key role of IL-1β in the pathophysiology of these syndromes has been confirmed by the dramatic response of CAPS patients to IL-1 blocking [58, 59].

Interestingly, the redox state of unstimulated CAPS monocytes displays some abnormalities. Unlike primary monocytes, which exhibit a redox equilibrium with low basal production of ROS and of antioxidants (Fig. 2B), and unlike THP-1 cells, which display an excess of antioxidants, monocytes with mutated NLRP3 have higher levels of ROS and up-regulation of antioxidant systems (Fig. 2C) in the absence of PAMP stimulation and IL-1β expression. This activated redox response causes a faster antioxidant response to PAMP-induced ROS, which results in a fastened IL-1β secretion. In fact, not only do monocytes from these patients secrete more IL-1β than healthy donors, but they also display a dramatically accelerated kinetics of secretion in response to TLR agonist stimulation [50].

Differently from THP-1 cells and cultured monocytes, where the high level of antioxidants is paralleled by a low level of ROS, both before and after TLR triggering, CAPS monocytes also exhibit a strong production of ROS at baseline that is increased by TLR agonists. As a result, TLR-stimulated CAPS monocytes display signs of stress, such as mitochondrial alterations, and drop of antioxidants, i.e., oxidative stress [60]. CAPS monocytes react to PAMP-induced oxidative stress by slowing down protein synthesis, a common response of stressed cells. Consequently, production of cytokines downstream of IL-1, such as IL-1Ra and IL-6, is severely impaired.

This finding has some important implications. First, the deficiency of IL-1Ra allows the amplification of IL-1-mediated effects. This is well-evident in patients genetically deficient for IL-1Ra [61], where lack of IL-1Ra allows unopposed action of IL-1 with dramatic consequences. The low availability of IL-1Ra explains the strong inflammmatory symptoms exhibited by CAPS patients (also those cases where the increase in IL-1β secretion is modest) and the dramatic responsiveness to IL-1-blocking therapies compared with autoimmune patients [62], whose monocytes may well secrete more IL-1β than controls, but accompanied by high levels of IL-1Ra [60].

Secondly, the different levels of IL-6 secretion by CAPS and autoimmune monocytes could have a part in the different clinical features of autoinflammatory versus autoimmune diseases. In fact, in autoimmunity, the strong production of IL-6 may sustain the role of the adaptive immune response [62], whereas in CAPS, the concomitant deficiency of IL-6 and IL-1Ra gives reason for the marked predominance of innate immunity. In addition, the different rate of IL-6 production explains why agents targeting IL-6 are highly beneficial in some autoimmune diseases [63] but have no sustained effects in CAPS, which conversely, are highly responsive to IL-1 blockade [58, 59].

These results shed new light into the pathogenic mechanisms of CAPS, indicating that the decreased IL-1Ra and IL-6 production coupled with increased IL-1β secretion contributes to the disease phenotype. Remarkably, genetic and epigenetic factors participate in the development and progression of the disease (**Fig. 3**). The mutated NLRP3 increases inflammasome efficiency; the concomitant redox dysfunctions are responsible for the accelerated IL-1β secretion and the impaired production of downstream cytokines. In other words, the mutation and the conditions of cell stress, most likely secondary to the mutation, collude in generating a more severe disease phenotype.

Oxidative stress and in general, aberrant redox responses may be a consequence of a genetic defect that perturbs cellular homeostasis. In fact, redox alterations are found in several genetic diseases [64–68]. In some cases, the presence of misfolded proteins or protein aggregates is the cause of cell stress and of the consequent pathologic responses, including the redox distress. In others, the functional link between mutation and cell stress is less clear. This is the case of CAPS. Why and

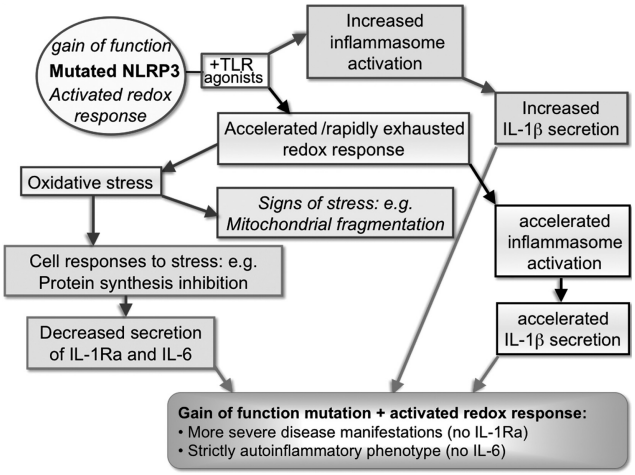


**Figure 3. Involvement of NLRP3 mutations and redox alterations in the pathophysiology of CAPS.** The expression of mutated NLRP3 not only increases the inflammmasome efficiency with increased IL-1$\beta$ secretion (gray arrows) but also causes cell stress, resulting in activated redox response (see text). Because of this altered redox state (see Fig. 2C), cells expressing the mutated gene display an impaired redox response to TLR agonists that results in accelerated IL-1$\beta$ processing and secretion (black arrows). However, the redox response has an early failure, and cells undergo oxidative stress. This is evidenced by stress signs, such as mitochondria alterations, and by cell responses to stress, including down-modulation of translation (brown arrows). As a consequence, production of cytokines downstream of IL-1, such as IL-1Ra or IL-6, is impaired (dotted line arrows). Therefore, the genetic defect (that increases the efficiency of inflammasome activation) colludes with the redox dysfunctions (responsible for the accelerated inflammasome activation and the impaired production of IL-1Ra), resulting in a more severe disease phenotype. Moreover, the impaired production of IL-6 coupled with the increased IL-1$\beta$ secretion gives reason for the main implication of the innate immunity (dash-point line arrows) [60].

how NLRP3 mutations cause cell stress are unclear, as *NLRP3* is expressed at a low level by resting monocytes, and misfolding has not been reported, and deserves further investigations. Nevertheless, increased ROS levels also characterize monocytes from patients with *NLRP12* mutations, responsible for a mild autoinflammatory disease [69], and patients affected by TNFR-associated periodic syndrome, an autoinflammatory syndrome linked to mutated *TNFR1* [70], suggesting that loss of redox homeostasis is a hallmark of monocytes from monogenic autoinflammatory syndromes expressing mutated proteins.

## CONCLUSIONS AND PERSPECTIVES

As corroborated by a large body of evidence, redox signaling is a crucial mechanism of inflammasome activation and IL-1$\beta$ processing and secretion. However, the mechanistic link between redox reactions and inflammasome oligomerization is still missing. Moreover, many open questions remain concerning the inflammasome functions. For instance, although it is clear that maturation and secretion of IL-1$\beta$ are associated, as IL-1$\beta$ is secreted immediately after cleavage, how inflammasome-mediated processing licenses the externalization of the mature cytokine is so far, unknown. Moreover, activated NLRP3 inflammasome (and enzymatically active caspase-1) was recently shown to be essential for secretion, not only of IL-1$\beta$ and IL-18 but also of other leaderless secretory proteins [71], including high-mobility group protein B1 [72] and FGF2, although these proteins are not cleaved by caspase-1. Which are the components of the molecular machinery driving the secretion of leaderless proteins? How are they regulated by NLRP3 inflammasome and caspase-1? How is the redox response involved? Answering these questions will hopefully clarify new mechanisms by which inflammasomes contribute to host defense and immunity. Finally, given the emerging role of NLRP3 inflammasome in many chronic diseases, understanding the molecular bases of redox-mediated inflammasome activation may hold important insights in developing future strategies of therapeutic intervention.

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KEY WORDS:
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