GREMI Prize 2013 - PhD Thesis Award ex-aequo:
Mechanisms of activation of the NLRP3 inflammasome by micro- and nano-particles in a murine model of lung inflammation

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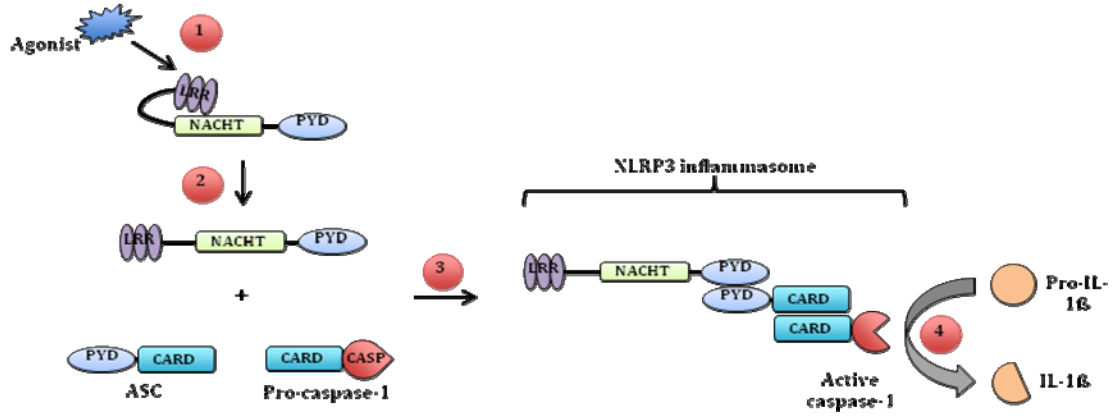
Inflammation is a set of reactions generated by the organism in response to insult or damage. This insult can be induced by pathogens, which are detected by exhibiting particular patterns (PAMPs for Pathogen-Associated Molecular Patterns) such as lipopolysaccharides (LPS), but also, can occur in absence of these pathogens. This process is referred to as sterile inflammation, and is triggered by the liberation or the production of danger signals or DAMPs (Damage-Associated Molecular Patterns) such as ATP, uric acid or cholesterol. The detection of PAMPs or DAMPs by cells (immune or not) requires PRRs (Pattern Recognition Receptors), which activate innate immunity. The most well-known are the TLR (Toll-Like Receptor) family, inserted into the cellular or endosome membrane. There is also another equally important family: the NLRs (Nod-Like Receptors), which are cytoplasmic. The activation of some of them allows for the establishment of an intracellular multiprotein complex called the inflammasome. The most studied is the NLRP3 inflammasome. It is characterized by numerous and different agonists which confer its key role not only in the detection of invasive pathogens but also of non-microbial aggressions, particularly by particles. The NLRP3 inflammasome has been recently involved in several metabolic disorders and in autoimmune diseases.



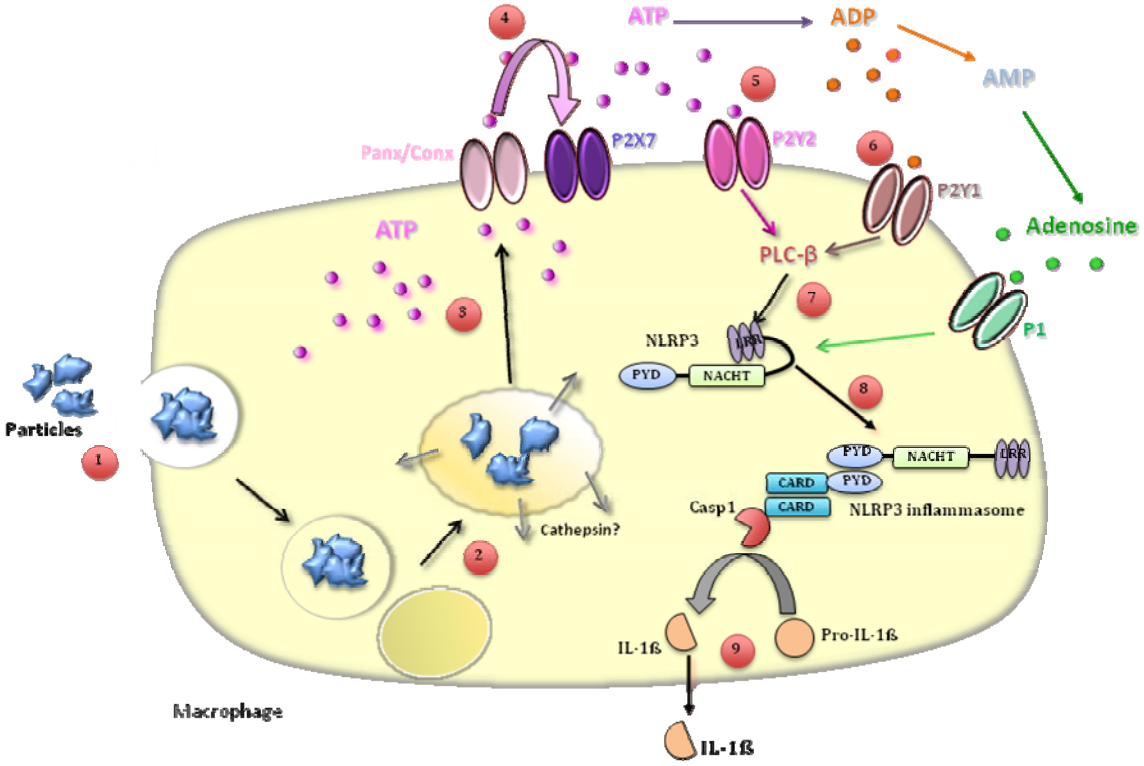
NLRP3 inflammasome activation

The cytoplasmic NLRP3 receptor is activated by an agonist (1) and then changes conformation (2). It is thus able of recruit an adaptator molecule, ASC, through domain-domain interactions. ASC in turn recruits the effector protein caspase-1. This whole complex is called the NLRP3 inflammasome (3). The NLRP3 inflammasome allows pro-caspase-1 activation, which will cut the pro-interleukin-1ß (pro-IL-1ß) into a mature and inflammatory cytokine, IL-1ß.

During my thesis, I have been interested in the mechanisms of activation of the NLRP3 inflammasome by nano- and micro- particles in a lung inflammation model. Repeated aggressions of pulmonary epithelium may be involved in the exacerbation of inflammatory diseases such as asthma and several particles are responsible for irreversible diseases such as pulmonary fibrosis. Thus, these diseases in a world where people have increased exposure to pollution, constitutes a major of public health problem.

First, we demonstrated *in vitro* that cells stimulated with microparticles of silica, alum (used as adjuvant in vaccines) or uric acid, actively release ATP via hemichannels. This extracellular ATP (eATP), more than the particles themselves, is responsible for the NLRP3 inflammasome activation, since the complete degradation of eATP abolishes cellular IL-1 ß production. Second, we studied the effects of silica and titanium

nanoparticles, good candidates because of their incorporation in a variety of domains such as buildings, food and cosmetics. In mice, inhalation of high doses of these nanoparticles provokes acute lung inflammation, marked by an influx of neutrophils in the airways and the production of inflammatory molecules such as cytokines (whose IL-1ß) and chemokines in the lung. This inflammation is, from moderately to considerably, reduced in mice deficient in NLRP3 inflammasome components or mice deficient in IL-1ß receptor. These results demonstrate the importance of the NLRP3 inflammasome and of IL-1ß in this inflammatory model. As previously described for microparticles, silica and titanium nanoparticles induce the active release of ATP by several cellular lineages. But, surprisingly, the degradation of eATP exacerbates nanoparticle-induced lung inflammation. We demonstrate in this model that ATP metabolites, in particular ADP and adenosine, are highly inflammatory via the activation of several purinergic receptor signaling pathways (manuscript in redaction).



Model of NLRP3 inflammasome activation by particles in macrophages

Particles are internalized by phagocytosis (1). After lysosomal fusion, the phagolysosomal membrane is destabilized, which induces cathepsin release into the cytosol (2). This precedes extracellular ATP (eATP) release through pannexin/connexin hemichannels (3). High concentrations of eATP can activate the purinergic receptor P2X7 to amplify ATP release (4). ATP, but also its metabolite ADP can bind to purinergic receptor P2Y2 (5). ADP is also a ligand for the P2Y1 receptor (6). In the same way, ADP is then degraded by ectoenzymes into AMP and then adenosine, which is the agonist of purinergic receptors P1. Downstream, P2Y1, P2Y2 and P1 receptors activate the NLRP3 receptor; the mechanism has not yet been elucidated (7). The established NLRP3 inflammasome (8) activates caspase-1, which then matures IL-1ß (9).

With aggressions of lung epithelium particles are responsive of inflammations. Our studies show that a two-step model can summerize particle-induced inflammation in the following:

- Release of a danger signal, ATP, which, directly or indirectly by this metabolites such as ADP or adenosine, activates several purinergic receptors and by consequence the NLRP3 inflammasome
- Activation of the NLRP3 inflammasome responsible for IL-1ß maturation.