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Complement activation by cholesterol crystals triggers a subsequent cytokine response

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ABSTRACT

In the host a diverse collection of endogenous danger signals is constantly generated consisting of waste material as protein aggregates or crystalline materials that are recognized and handled by soluble pattern recognition receptors and phagocytic cells of the innate immune system. These signals may under certain circumstances drive processes leading to adverse inflammation. One example is cholesterol crystals (CC) that accumulate in the vessel wall during early phases of atherogenesis and represent an important endogenous danger signal promoting inflammation. CC is recognized by the lectin- and classical pathways of the complement system resulting in activation of C3 and C5 with release of inflammatory mediators like the potent C5a fragment. Complement activation by CC leads to crosstalk with the NLRP3 inflammasome-caspase-1 pathway and production of IL-1β. Neutralization of IL-1β may have beneficial effects on atherosclerosis and a large clinical trial with an IL-1β inhibitor is currently in progress (the CANTOS study). However, upstream inhibition of CC-induced inflammation by using a complement inhibitor may be more efficient in treating atherosclerosis since this will block initiation of inflammation processes before downstream release of cytokines including IL-1β. Another therapeutic candidate can be broad-acting 2-hydroxypropyl-β-cyclodextrin, a compound that targets several mechanisms such as cholesterol efflux, complement gene expression, and the NLRP3 pathway. In summary, emerging evidence show that complement is a key upstream player in the pathophysiology of atherosclerosis and that therapy aiming at inhibiting complement could be effective in controlling atherosclerosis.

1. Introduction

1.1. Damage associated molecular patterns and altered-self

Inflammation is essential for elimination of invading microbes and tissue repair. In response to infection, a cascade of events leads to the recruitment of innate immune cells such as neutrophils and macrophages that phagocyte and eliminate the intruders. The innate immune system will also result in the production of immune effectors such as cytokines that lead to the activation of the adaptive immune system. Inflammation is also involved in wound repair after tissue injuries such as after trauma and ischemia-reperfusion. Such inflammation that occurs in the absence of pathogens has been termed sterile inflammation. As for the inflammation caused by pathogens, unresolved sterile inflammation can be detrimental to the host leading to chronic inflammation in many diseases. The production of reactive oxygen species (ROS), pro-inflammatory cytokines, proteases and growth factors by immune competent cells may lead to severe tissue damage with increased immune cell

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infiltration. This will eventually result in the formation of a necrotic core that triggers furthermore inflammation.

Various particles can incite inflammation, including those whose composition is inorganic such as silica oxide and asbestos (Mossman and Churg, 1998), or calcium pyrophosphate (Ea and Liote, 2004), and organic such as monosodium urate (MSU) (Nuki and Simkin, 2006), cholesterol (Hansson and Hermansson, 2011), or amyloid-β (Weiner and Frenkel, 2006). The sterile inflammatory response triggered by these particles does not appear to be a host defense mechanism as it is the case with pathogens, but rather "a cry for help" by immune cells (Matzinger, 2002). In case of aberrant crystallization of particles inside the body (e.g.; cholesterol or MSU), phagocytosis of these can go wrong resulting in the damage of the lysosomal membrane and subsequent leakage of lysosomal enzymes (e.g.; cathepsin B), which damage cellular organelles such as the mitochondria (Franklin et al., 2016).

What seems to be a common denominator is that the inflammatory response generated fails to clear them, and the damage caused by sterile inflammation can lead to severe inflammatory diseases. Inhalation of asbestos and silica particles leads to asbestosis and silicosis, respectively, with continuous activation of alveolar macrophages and consequently pulmonary interstitial fibrosis (Mossman and Churg, 1998). Sterile inflammation is also involved in diseases such as gout where the deposition of MSU in the joints causes neutrophil infiltration and severe inflammation (Busso and So, 2010). Severe progressive atherosclerosis is linked to sterile inflammation caused by accumulation of cholesterol crystals (CC) in the arterial wall (Duewell et al., 2010). Other severe pathologies include Alzheimer's disease where deposition of amyloid-β in the central nervous system leads to activation of a pathological innate immune response with degeneration of brain tissue (McGeer and McGeer, 2001), and type 2 diabetes where saturated fatty acids and obesity-related metabolites trigger chronic inflammation (Grant and Dixit, 2013).

In addition to sterile particulates discussed above, tissue damage can be caused by molecules released by necrotic cells and their debris. Necrotic cells stimulate an acute inflammatory response, with massive infiltration of neutrophils in tissue (Chen et al., 2007). Under cellular stress or tissue injury, molecules are released by dying cells in the extracellular space where some of the released molecules trigger an inflammatory response. This is typical for purine metabolites such as ATP (Bours et al., 2006) or chromatin associated high-mobility group complex (HMGB1) (Scaffidi et al., 2002) or heat shock proteins (HSPs) (Quintana and Cohen, 2005).

The infiltration of neutrophils in the tissues will increase the magnitude of injury similar to the diseases caused by altered-self particles. This is well described in ischemia and reperfusion injury such as in myocardial infarction, stroke or acute kidney injury. In all these conditions, the restoration of blood flow causes severe tissue injury as a response to necrotic cells (Eltzschig and Eckle, 2011).

1.2. Detection of altered-self

The same mechanisms involved in the recognition of microorganisms are involved in the induction of sterile inflammation. The innate immune system uses a range of immune sensors called pattern recognition receptors (PRRs), also termed pattern recognition molecules (PRMs), to monitor and resume inflammation. PRRs detect microbial conserved substances, so called pathogen-associated molecular patterns (PAMPs) and endogenous molecules released from cell- and tissue damage, named damage associated molecular patterns (DAMPs) (Janeway, 1989).

Several families of PRRs have been identified and their subcellular localization varies and reflects their various biological roles. The Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are located on the plasma membrane and on internal membranes (e.g., endosome, lysosome, or endoplasmic reticulum). The cytosol contains receptors such as the Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and Nucleotide binding oligomerization domain receptors NOD-like receptors (NLRs). The complement system is another pattern recognition system that operates both extracellularly (Kohl, 2006) and intracellularly (Liszewski et al., 2013). The sensing of PAMPs or DAMPs by PRRs leads to the transcription of pro-inflammatory cytokines, chemokines, type I interferons (IFNs), or antimicrobial peptides and proteins that modulate inflammatory signaling (Takeuchi and Akira, 2010).

2. The complement system

Complement was originally viewed only as a non-specific, first line defense mechanism against microbial intruders. However now, the role of complement beyond elimination of microbes is widely recognized. By sensing danger and relaying danger signals as well as triggering elimination of cell debris, complement plays a major role in homeostasis. The complement system is activated by three pathways (Fig. 1): the classical- (CP), the lectin- (LP), and the alternative pathway (AP), whose components mediate immune surveillance and cell homeostasis by cross-talking with other parts of the immune system, as well as with adaptive immunity, and a number of other biological systems (e.g. hemostatic, neuroendocrine and metabolic). To protect against self-attack, a number of regulatory plasma and membrane-bound regulatory proteins have evolved. These factors act to restrict complement activation, and anything that changes the balance between complement activation and regulation may disrupt homeostasis.

The PRM C1q in the CP activates complement by recognizing molecules such as immunoglobulins (IgM or IgG) bound to their antigen, or pentraxins (e.g., C-reactive protein; CRP) as well as distinct structures on microbial or apoptotic cells. The LP shares some similarities with the CP but is initiated through mannose-binding lectin (MBL), ficolins (FCN-1, FCN-2 and FCN-3) and collectins (CL-10 and CL-11) which recognize various carbohydrate structures (Garred et al., 2010; Hansen et al., 2016). Furthermore, LP uses associated serine proteases (MASPs) instead of C1r and C1s utilized by the classical pathway to exert downstream complement activation (Yongqing et al., 2012). In contrast to the CP and LP, the AP is spontaneously activated by hydrolysis of the C3 thioester and triggered by foreign surfaces lacking complement regulatory proteins. Activation of all three pathways converge at C3 and subsequently C5. C3 and C5 convertases are enzyme complexes that cleave C3 into C3a and C3b, and C5 into C5a and C5b, respectively. C3b binds covalently to the surface and is cleaved to iC3b, both serving as opsonins. Formation of C5b leads to binding of C6, C7, C8 and C9, the terminal complement complex (TCC), which may either be inserted into a membrane as the membrane attack complex (MAC) or build up in the fluid-phase as a soluble complex (sC5b-9). MAC can form a lytic pore which kills some Gram-negative bacteria and can lyse cells, particularly red blood cells, but alternatively makes a sublytic pore which activates cells instead of lysing them (Tegla et al., 2011).

Receptors of complement fragments are expressed on almost all immune cells. Complement is therefore not only an important danger sensor able to recognize harmful structures, but also a danger transmitter able to translate danger signals into suitable immune responses. Cleavage products of C3 (e.g., C3a, C3b, iC3b and C3dg) have the ability to relay signals that alarm cells from the innate and adaptive immunity through interaction with specific receptors (e.g. C3aR, CR1, CR2, CR3, CR4 and CRIg). C5a is a highly potent chemoattractant that binds to C5aR1, triggering pro-inflammatory cytokines, and to C5aR2 complement suggested to attenuate the pro-inflammatory effect (Arbore et al., 2016). A number of solu-

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