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Poxviruses Aren't StuPYD

Pathogens utilize many strategies to dampen the host inflammatory response. In this issue of *Immunity*, a report by Johnston and colleagues reveals a poxvirus strategy that inhibits the inflammasome, arresting secretion of interleukin-1-related cytokines, thus silencing key alarms that mobilize host defenses.

The host-pathogen relationship is complex, and in most cases a balance exists between host control and pathogen escape from immune defenses. The poxviruses in particular have evolved numerous strategies to inhibit or dampen the host immune response to infection (Seet et al., 2003). In their work, Johnston and colleagues describe the M13L protein that is encoded by a rabbit poxvirus (myxoma virus) and that blocks the production of interleukin (IL)-1 family cytokines from infected cells (Johnston et al., 2005). M13L contains a pyrin domain (PYD), a member of the “death-fold” family that is composed of six tightly packed α helices to generate a Greek key fold, which can interact specifically with a cellular PYD-containing protein called ASC-1 (apoptosis-associated speck-like protein containing a caspase recruitment domain) (Masumoto et al., 1999). ASC-1 is a component of the “inflammasome,” a multiprotein complex located in the cytosol that is responsible for activation of proinflammatory caspases (cysteine-based aspartic acid-specific proteinases, caspase-1, -4, and -5 in humans). Active caspase-1 and/or caspase-5 proteolytically process proIL-1 β (and IL-18) to its active form in the cytosol (Martinon and Tschopp, 2004). Johnston et al. show that M13L-PYD inhibits the activation of procaspase-1 and subsequent secretion of IL-1 β and IL-18 from myxoma-infected cells (Johnston et al., 2005). The authors establish the importance of M13L-PYD in the course of myxoma infection by using a mutant virus deleted in the PYD domain of the M13L gene. This mutant virus caused a limited infection that failed to spread from the initial site of inoculation, demonstrating that the M13L protein contributes to the virulence in this host and underscoring the importance of the IL-1 family in mounting effective defenses.

Tight regulation of the production of proinflammatory cytokines is required to maintain the homeostasis of host tissues. Insufficient control of IL-1 production through deregulation of the inflammasome can lead to

inflamed tissues and organ damage. There are several types of these autoinflammatory diseases in humans (e.g., Muckle-Wells syndrome, familial cold urticaria, and familial Mediterranean fever) where mutations in key components of the inflammasome are thought to contribute to disease (Martinon and Tschopp, 2004). NALP3 promotes inflammasome assembly and is frequently mutated in its NACHT/NOD domain, leading to increased “signal-independent” activation of IL-1 β processing (Agostini et al., 2004). Most components of the inflammasome (e.g., NALP, ASC-1, caspases, and others) encode multiple protein-interaction domains (e.g., PYD, CARD, and NOD/NACT) that promote homotypic interactions between molecules that contain similar domains, forming the scaffold of the inflammasome. For instance, ASC-1 contains both a PYD and a CARD and can function as a molecular bridge through its interaction with the PYD of various NALP and the CARD of caspase-1 (see Figure 1 inset).

Unlike most inflammasome components, M13L-PYD contains a single PYD domain at its N terminus and can interact with ASC-1 through a PYD-PYD association (Johnston et al., 2005). This association of M13L-PYD may disrupt the ability of ASC-1 to form the bridge between caspases and various NALP, resulting in the inhibition of caspase-1 activation. A cellular PYD-only-protein (POP1) that interacts with ASC-1 has been described, but POP1 does not appear to arrest IL-1 β production (Stehlik et al., 2003), suggesting that M13L-PYD utilizes a distinct mechanism of action from POP1. One possible model is shown in Figure 1, wherein M13L-PYD disrupts the formation of a NALP3-containing inflammasome through its interaction with ASC-1. However, several molecularly distinct inflammasomes exist, each composed of a particular combination of death-fold-containing adaptor proteins (Martinon and Tschopp, 2004). The exact nature of the inflammasome that is induced by myxoma infection, and consequently inhibited by M13L-PYD, is currently unknown, but its future identification will certainly add mechanistic insight to how M13L-PYD functions to inhibit inflammation.

How does myxoma virus infection activate the inflammasome? Although numerous studies have dissected the molecular aspects of protein-protein interactions that regulate inflammasome assembly, to date the number of known physiological ligands/stimuli that catalyze the assembly are limited. Bacterial muropeptide components of peptidoglycans bind directly to the leucine-rich

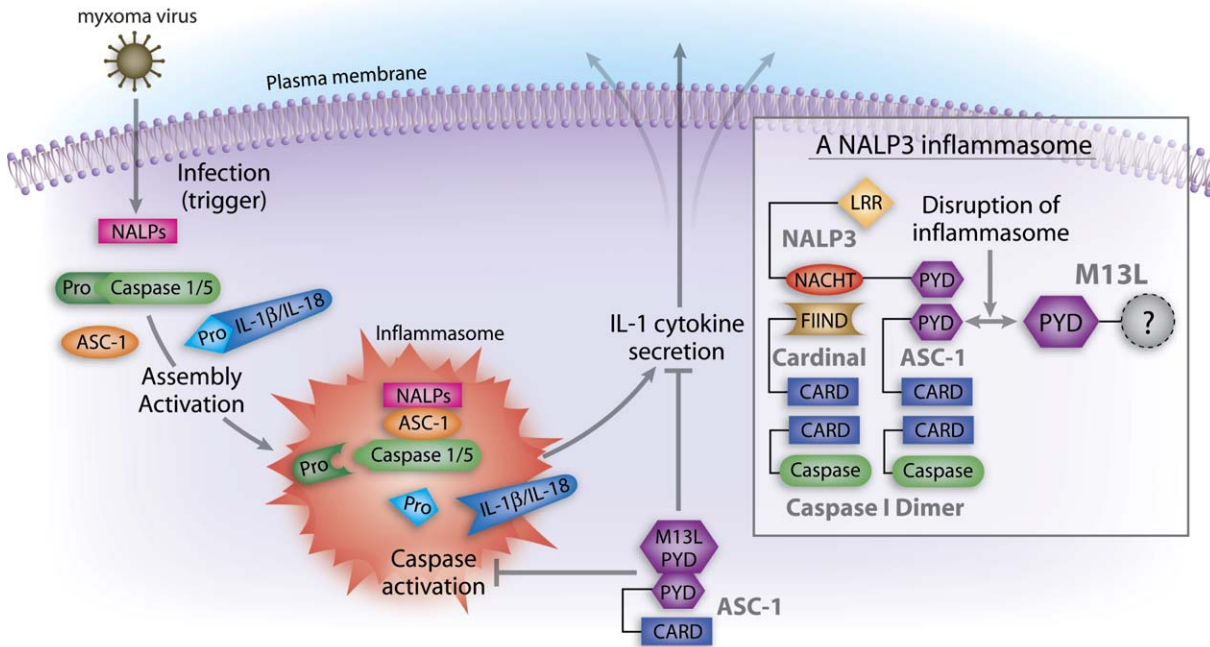


Figure 1. Poxvirus Disruption of the Inflammasome

Myxoma virus (a rabbit poxvirus) encodes a protein, M13L-PYD, that blocks production of interleukin (IL-1) family cytokines by inhibiting activation of the cytoplasmic “inflammasome” (Johnston et al., 2005). M13L is composed of an N-terminal pyrin domain (PYD) and a C-terminal domain of unknown function, and it interacts with a cellular PYD domain-containing protein (ASC-1) to inhibit inflammasome function. The exact molecular components of the myxoma-induced inflammasome are not known, and neither is the precise ligand (i.e., trigger) that catalyzes its formation. For descriptive purposes, a NALP3 (also called cryopyrin, Pypaf1, CIAS1)-containing inflammasome is depicted (see inset box), with the specific protein domains shown in similar colors. Like domains interact between the various inflammasome components to catalyze assembly. The FIIND domain of Cardinal (also called TUCAN, CARD8, NDDP1) is encoded within NALP1 (Martinon and Tschopp, 2004). CARD, caspase recruitment domain; LRR, leucine-rich repeat; FIIND, F-interacting domain; NALP, NACHT-LRR-PYD domain-containing protein.

repeats (see Figure 1) of NALP3, which resides in the cytoplasm, initiating inflammasome assembly/activation independent of Toll-like receptor signaling (Martinon et al., 2004). Nuclear factor- κ B activation through cytoplasmic recognition of bacterial muopeptides by members of the NOD family has also been reported (Girardin et al., 2003). These studies demonstrate that several inflammatory pathways are regulated by direct recognition of pathogen components in the cell cytoplasm, the exclusive site of poxviral replication. Johnston and colleagues demonstrate that infection of a human monocyte cell line with a mutant myxoma virus deleted for the PYD domain of M13L was a strong activator of caspase-1 and of the secretion of IL-1 β and IL-18, as compared to the muted response seen with wild-type virus (Johnston et al., 2005). These results suggest that myxoma may contain an as yet uncharacterized ligand for the inflammasome activation that is suppressed by M13L-PYD function, although it is also possible that myxoma infection induces production of a cellular ligand that activates the inflammasome.

In addition to the function that M13L-PYD plays in inhibiting inflammasome activation, the M13L-PYD myxoma virus mutant replicated very poorly in cultured rabbit lymphocytes and monocytes, while replication in fibroblasts was normal (Johnston et al., 2005). Consistent with this result, fewer infected cells were found circulating in the peripheral blood from rabbits infected with the mutant virus, suggesting that the dissemination of virus was

impeded. Indeed, decreased numbers of secondary lesions in the skin were observed in rabbits infected with the M13L-PYD mutant. However, the precise reason for the abortive replication in hematopoietic cells by myxoma virus carrying a mutated M13L-PYD is still unclear.

The natural host for myxoma virus is the South American rabbit, *Sylvilagus brasiliensis*, in which it has effectively adapted to cause a limited, benign infection. However, when introduced into a foreign host such as the European rabbit, *Oryctolagus cuniculus*, myxoma virus causes a severe, systemic infection, a feature historically employed as a biological agent for control of *Leporidae* in Australia (Zuniga, 2002). This (un)intentional jump to a new host species is a classic example of the tenuous balance between a pathogen’s virulence factors and host defense systems. Surprisingly, although the large DNA genome of myxoma virus harbors a hefty arsenal of immunomodulating genes, deletion of a single one such as M13L-PYD attenuates virulence. Indeed, deletion of one of several other myxoma virus inflammatory modulators such as M-T2, the tumor necrosis factor inhibitor, also limits virulence in the nonadapted host (Seet et al., 2003). Consequently, the myxoma system provides an extremely powerful model for analyzing the contributions of various immune-evasion genes to the pathogenesis of poxvirus infections and host responses, recognizing the caveat that not all poxviruses encode the same immunomodulatory proteins. Nonetheless, poxviruses are highly instructive in identifying

key regulatory components of the host's inflammatory and immune systems, with significant collateral benefit for other inflammatory diseases. Specifically, this investigation by Johnston et al. highlights the importance of a specific target within the inflammasome, the PYD domain, and its consequential role in cytokine production and inflammation.

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Selected Reading

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