CATERPILLER (NLR) Family Members as Positive and Negative Regulators of Inflammatory Responses

John D. Lich¹ and Jenny P.-Y. Ting¹

¹Department of Microbiology–Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina

One of the most important advances in human immunology in the last decade has been the characterization of evolutionarily conserved molecular mediators important in controlling innate immunity. A prime example of this is the discovery of the mammalian Toll-like receptor family. Toll molecules were first discovered in *Drosophila* and were found to protect the organism from fungal infection. In mammals, Toll-like receptors respond to a wide variety of microbial products and serve as a bridge between innate and adaptive immunity. In the last 4 years, another important family of molecules has been discovered, and it is evolutionarily conserved from plants to humans. This family was first christened CATERPILLER by our laboratory, and is also known as NBD-LRR or NLR. CATERPILLER family members have rapidly gained prominence as important regulators of inflammatory responses to pathogens and their products. This article discusses some of the members of this family and their role in human disease.

Keywords: inflammation; innate immunity

The evolutionary conservation of gene families predicts pivotal roles for such families in host survival. This is evident in the recent discovery of two families of genes that encode important mediators of innate immunity and provide the first line of defense against pathogens. One of these families is the mammalian Toll-like receptor (TLR) family. These genes were predicted to exist based upon the discovery of the Toll receptor in *Drosophila* (1, 2). In *Drosophila*, Toll controls the host response to fungi by regulating the expression of the antifungal peptide gene, drosomycin. Like Toll, mammalian TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic domain homologous to that of the IL-1 receptor. Both *Drosophila* Toll and TLRs serve as pattern recognition molecules and signal through the nuclear factor (NF)- κ B pathway to mediate innate immune responses to a wide range of pathogen-derived products. Furthermore, TLRs are also crucial for the activation of key constituents of the adaptive immune system, such as dendritic cells and lymphocytes (3–5).

More recently, we and others have discovered a second family of evolutionarily conserved genes that spans the animal and plant kingdoms. Proteins encoded by these genes are marked by the presence of a conserved nucleotide-binding domain (NBD) followed by C-terminal LRR domains (6). Proteins bearing these structural domains were first discovered in plants, and represent the most prevalent subfamily of plant disease resistance (R) proteins. R proteins mediate plant host responses to a wide

range of pathogens, and likely represent the oldest members of the NBD-LRR containing proteins (7). These proteins can be divided into two major groups: those with N-terminal coiled-coil structures, and those with N-terminal TLR/IL-1 receptor domains.

Although the plant genome encodes hundreds of NBD-LRR proteins, mammalian species only encode 20–30 such genes. We first christened the human NBD-LRR proteins as the CATERPILLER (caspase activation and recruitment domains [CARD], transcription enhancer, R [purine]-binding, pyrin, lots of leucine repeats) family. Others have named these proteins nucleotide oligomerization domain (NOD)-LRR or NLR (NACHT [NAIP, CIITA, HET-E, and TP1]-LRR or NBD-LRR) (8–10). Although all genes within the CATERPILLER family encode NBD-LRR structures, the N termini are variable, and include pyrin domains, CARD, acid transcriptional activation domains, and baculovirus inhibitory repeat domains.

CATERPILLER PROTEINS AND DISEASE ASSOCIATION

A natural manifestation of the importance of any gene is its linkage to human disease. Several CATERPILLER family members are genetically linked to known immunologic disorders. Major histocompatibility complex (MHC) class II transcriptional activator (CIITA) is the founder of the CATERPILLER family. This protein functions as the master regulator of both cytokine-induced and constitutive expression of MHC class II molecules, which present antigenic peptides to T cells (11, 12). Mutations in CIITA are linked to bare lymphocyte syndrome. This immunodeficiency disease is characterized by the absence of MHC class II molecules and results in a severe loss of adaptive immunity (13).

Nod1, the founding member of the CARD-containing subgroup of CATERPILLER genes, was originally described as an activator of caspase-9-mediated apoptosis and NF- κ B when studied in an overexpression system. It was later found to be an important regulator of cellular responses to the bacterially derived mur peptide, GM-TriDAP (GlcNAc-MurNAc tripeptide mur peptide) (14, 15). Mutations in Nod1 are associated with susceptibility to asthma and sarcoidosis (16, 17).

Mutations in Nod2, a CATERPILLER gene closely related to Nod1, have also been linked to human disease. Such diseases include the inflammatory bowel disorder, Crohn's disease, and Blau's syndrome, a familial granulomatous disease characterized by inflammation of the eyes, joints, and skin (18–20). Similar to Nod1, the gene product encoded by Nod2 is required for the cellular response to the bacterially derived molecule, muramyl dipeptide.

Finally, mutations in pyrin-containing CATERPILLER protein NALP3 (NACHT domain-, leucine-rich repeat-, and pyrin-containing protein)/cryopyrin are associated with several clinical autoinflammatory syndromes. These include familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and chronic infantile neurologic cutaneous and articular syndrome, also known as neonatal-onset multisystem inflammatory disease (14–18, 21–25). As discussed subsequently here, NALP3 is required for cellular responses to a variety of microbial-derived products.

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Correspondence and requests for reprints should be addressed to Jenny P.-Y. Ting, Ph.D., Department of Microbiology–Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295. E-mail: jenny_ting@med.unc.edu

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THE EMERGING ROLE OF CATERPILLER PROTEINS IN IL-1 β PRODUCTION

IL-1 β is a highly potent proinflammatory cytokine and, as such, its regulation must be tightly controlled. Several CATERPILLER proteins have been shown to play a key role in the post-transcriptional regulation of IL-1 β processing and release. NALP1/DEFCAP (death effector filament-forming CED4-like apoptosis protein)/CARD7 (26–28) was the first CATERPILLER protein shown to perform this important function. Using a cell-free reconstitution system, it was demonstrated that NALP1 assembles a multiprotein IL-1 β processing complex, termed the “inflammasome,” which is composed of ASC-1 (the apoptotic speck-containing protein with a CARD), caspase-1, caspase-5, and pro-IL-1 β (29). Later, this same group demonstrated that both NALP2 and NALP3 can also form inflammasome complexes and mediate the production of IL-1 β (30).

The importance of the NALP3 inflammasome in the production of IL-1 β has recently been highlighted by several reports using knockout mice. In these studies, it was shown that the NALP3 inflammasome mediates the production of IL-1 β in response to a variety of stimuli, including LPS/ATP, bacterial RNA, toxins, and gout-associated uric acid crystals (31–34). In addition, a more recent report demonstrated that NALP3 is required for IL-1 β production in response to double-stranded RNA and viral infection. Taken together, these data suggest that NALP3 is important for IL-1 β secretion in response to pathogen-derived molecules of both bacterial and viral origin (35).

The role of CATERPILLER proteins in the regulation of IL-1 β synthesis has recently been extended to IL-1 β -converting enzyme protease-activating factor (IPAF) (36, 37). In these studies, macrophages derived from IPAF knockout mice failed to produce IL-1 β in response to *Salmonella*-derived flagellin. This response did not require TLR5, a sensor molecule for extracellular flagellin, demonstrating that IPAF responds to the presence of this pathogen-derived protein within the cytoplasm.

Although IPAF, Nod1, Nod2, and NALP3 are required for cellular responses to distinct pathogenic products, it is important to note that there is no evidence demonstrating that these CATERPILLER proteins directly bind these molecules. Thus, it is more appropriate to consider these CATERPILLER proteins as agonists of host immune responses or pathogen sensors rather than receptors.

MONARCH-1 AND THE REDUCTION OF INFLAMMATORY RESPONSES

Although the above description primarily focuses on CATERPILLER proteins that positively regulate inflammatory or adaptive immune responses, we and others have found proteins in this family that negatively control inflammatory responses. The following will focus on one of these proteins, Monarch-1 (also known as PYPAF7 or NALP12).

Evidence that Monarch-1 functions as a negative regulator of inflammation has been provided by several different model systems. However, the most compelling results were obtained in the human monocytic leukemia cell line, THP-1, in which Monarch-1 expression was reduced through RNA silencing. When these cells are stimulated through proinflammatory receptors, including TLRs or the tumor necrosis factor (TNF) family receptor, CD40, they produce elevated levels of cytokines and chemokines (38, 39). Furthermore, in reciprocal experiments, THP-1 cells expressing elevated levels of Monarch-1 produce significantly less cytokine than wild-type cells, thus supporting a negative regulatory role for Monarch-1.

Biochemical studies demonstrate that Monarch-1 suppresses inflammation by targeting the NF- κ B pathway. NF- κ B represents a family of transcription factors that operate as a critical point of control for inflammatory responses. Activation of NF- κ B can occur through two distinct mechanisms, referred to as the canonical and noncanonical pathways (40). The canonical pathway is triggered immediately after cell stimulation, and can be mediated by a number of upstream kinases. Activation of this pathway results in the production of early proinflammatory cytokines, such as IL-1 β , IL-6, and TNF- α . In contrast, the noncanonical pathway displays much slower kinetics and is uniquely dependent upon the upstream kinase, NF- κ B-inducing kinase (NIK). In this alternative pathway, NIK associates with and induces the cleavage of p100 to its active form, p52, leading to the expression of a distinct subset of inflammatory genes (41).

Studies in our laboratory demonstrate that Monarch-1 does not affect canonical NF- κ B, but instead suppresses activation of the noncanonical pathway. Interestingly, the ability of Monarch-1 to inhibit the noncanonical pathway stems from its ability to associate with NIK and induce proteasome-mediated degradation of the kinase. This results in greatly reduced production of p52-dependent genes, including CXCR4, CXCL12, and CXCL13. Presumably, this prevents inappropriate activation of these p52-dependent genes during the initial stages of inflammation. As the inflammatory response continues, Monarch-1 expression fades (38), and this allows the alternative pathway to proceed and properly orchestrate a protective response.

In addition to negatively affecting noncanonical NF- κ B, which is typically triggered by TNF family receptors, Monarch-1 also functions in a negative capacity downstream of TLRs. Upon stimulation, TLRs recruit the serine/threonine kinase, IL-1 receptor-associated kinase (IRAK-1). At the receptor complex, IRAK-1 becomes hyperphosphorylated, allowing it to operate in downstream signaling pathways. Monarch-1 associates with activated forms of IRAK-1 after TLR stimulation, and this leads to a striking reduction in hyperphosphorylated forms of IRAK-1 (38). The mechanism by which Monarch-1 performs this function is not clear. However, the results from our NIK studies have led to the hypothesis that hyperphosphorylated IRAK-1 is targeted for proteasome-mediated degradation. Indeed, in overexpression studies, the addition of proteasome inhibitors rescues hyperphosphorylated IRAK-1, suggesting a role for proteasome degradation in this process (J.D.L., unpublished observations). Current studies are underway to determine if Monarch-1 induces proteasome-mediated degradation of hyperphosphorylated IRAK-1 in monocytes.

The degradation of signaling molecules after cellular activation provides an effective mechanism for controlling inflammatory signaling pathways. Our results support a model in which stimulation of monocytes leads to the association of Monarch-1 with the active forms of the signaling proteins IRAK-1 and NIK (Figure 1). This interaction leads to rapid degradation of these proteins, thereby attenuating the inflammatory signaling. Other CATERPILLER proteins have been shown to associate with signaling proteins and suppress their ability to activate NF- κ B. For instance, Nod2 associates with transforming growth factor beta activated kinase 1 (TAK1) and inhibits the ability of TAK1 to activate NF- κ B (42). Similarly, NALP2 associates with ASC and prevents NF- κ B activation downstream of a number of activators (43). It remains to be determined, however, if these CATERPILLER proteins mediate NF- κ B suppression through proteolytic pathways.

CONCLUSIONS

This report briefly summarizes the recently described CATERPILLER gene family that encodes proteins that serve

Figure 1. Scheme depicting how Monarch-1 suppresses inflammatory signaling. Toll-like receptors (TLRs) and tumor necrosis factor family receptors (TNFRs) play a critical role in the inflammatory response. TLRs recruit the adaptor molecules, myeloid differentiation factor 88 (MyD88) and Tollip, which, in turn, recruit the kinase, IL-1 receptor–associated kinase (IRAK-1), to induce the activation of canonical nuclear factor (NF)– κ B. IRAK-1 becomes hyperphosphorylated, facilitating its dissociation from the receptor complex to mediate additional signaling pathways. One of the genes induced by the canonical pathway is NF- κ B2/p100, which functions in the noncanonical pathway. The noncanonical pathway is triggered after the activation of NF- κ B–inducing kinase (NIK). This results in processing of p100 to its active form, p52.

This alternative pathway induces a distinct set of genes and also regulates the expression of genes induced by the canonical pathway. Monarch-1 does not affect initial activation of the canonical pathway. However, Monarch-1 suppresses the noncanonical pathway by associating with NIK and inducing its degradation via the proteasome. Monarch-1 also associates with IRAK-1 and suppresses the accumulation of hyperphosphorylated forms of the kinase; however, it is unclear whether or not this also involves proteasome degradation.

a variety of crucial regulatory functions during inflammation and infection. Mutations in a significant number of these genes are directly linked to inflammatory disorders. Although some CATERPILLER proteins are clearly positive regulators of the inflammatory process, others are negative regulators, and prevent an overzealous response. Considering the necessity of fine tuning the inflammatory response during respiratory disorders like allergy and asthma, there is little doubt that these newly discovered proteins will prove important in the onset and control of such diseases.

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