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TAMpering with Toll-like Receptor Signaling

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Toll-like receptors (TLRs) provoke a profound inflammatory response during host defense and must be controlled in order to avoid autoimmune and inflammatory diseases. In this issue, Rothlin et al. (2007) uncover a complex negative feedback mechanism to limit TLR signaling involving the Tyro3/Axl/Mer (TAM) family of receptor tyrosine kinases, which induce expression of the inhibitory proteins SOCS1 and SOCS3.

Once unleashed, the innate immune response has the power to eliminate infections and provide instruction for the adaptive immune system to establish a memory response upon repeated exposure to the provoking pathogen. There has been remarkable progress toward the identification of receptors that recognize microbial components during the innate immune response. Such receptors include the Toll-like receptors (TLRs), the NOD-like receptors (NLRs), the RIG-I-like receptors (RLRs), and the C-type lectin receptors (CLRs) (Creagh and O'Neill, 2006; Brown, 2006). Activated TLRs induce a large number of immune and inflammatory proteins that are required for pathogen elimination. Because the ensuing inflammatory response has the potential to damage the host, multiple negative regulators modulate TLRs at various levels (Liew et al., 2005). An intriguing and potentially important mechanism of negative feedback is now reported by Rothlin et al. (2007) and implicates a hitherto poorly understood family of receptor tyrosine kinases termed the Tyro3/Axl/Mer (TAM) family (Lai and Lemke, 1991). The authors demonstrate that TAM receptors are induced by TLRs and then in response to the TAM ligands, Gas6 and ProS, TAM receptor signaling induces the suppressor of cytokine signaling-1 (SOCS1) protein and SOCS3, which act to limit TLR signaling. The elucidation of this mechanism provides us with a greater understanding of how TLR signaling is regulated and possibly points to new approaches to curtail TLR activation during inflammatory and autoimmune diseases or to enhance TLR activation in order to boost the efficacy of vaccination.

TAM receptor tyrosine kinases were first found in neurons, where Tyro3 was shown to have a role in neural adhesion. Subsequently it was shown that loss of function of the three receptors in a triple knockout mouse resulted in a profound dysregulation of the immune response, including massive splenomegaly and lymphadenopathy, lymphocyte infiltration into all tissues, high autoantibody levels, and broad spectrum autoimmune disease (Lu and Lemke, 2001). Splenomegaly is also evident in mice lacking only Mer. Importantly, these Mer-deficient mice were also shown to be hypersensitive to the TLR4 agonist lipopolysaccharide (LPS) (Camenisch et al., 1999). It was hypothesized that this hypersensitivity was due to loss of inhibitory TAM signaling in dendritic cells, which are key participants in innate immune responses and also in the activation of T lymphocytes. To test this notion, Rothlin et al. first measured numbers of dendritic cells in TAM triple knockout mice using the commonly used dendritic cell marker CD11c; they found markedly elevated numbers of dendritic cells compared to wild-type mice. These dendritic cells also expressed high levels of activation markers, including MHC class II and the costimulatory molecule B7.1. Dendritic cells from the triple knockout mice were hyperresponsive to LPS, producing elevated levels of the cytokines interleukin-6 and tumor necrosis factor relative to dendritic cells from wild-type mice. The hyperresponsiveness to LPS was progressively more severe in single, double, and triple knockout mice. The effect was not restricted to TLR4, as hyperresponsiveness was also seen to polyIC (a TLR3 ligand) and to CpG-DNA (a TLR9 ligand).

The authors then examined the effect of the TAM ligands Gas6 and ProS. Pretreatment of dendritic cells overnight with these ligands inhibited cytokine induction by LPS, polyIC, and CpG, as well as activation of the TLR signals NF-κB, ERK1/2, and p38 MAP kinase. The question then arose as to what might be the mechanism for this effect. The inhibitory effect was shown to require protein synthesis, and the authors therefore looked for candidates that were known to inhibit TLRs and that were inducible. The SOCS1 protein was an obvious candidate because it is induced by cytokine receptors. Furthermore, knockout mice reconstituted with T lymphocytes expressing SOCS1 develop an autoimmune phenotype very similar to that of TAM triple knockout mice (Hanada et al., 2003). SOCS3 was also tested, and both were shown to be induced in dendritic cells by TAM activation, whereas several other negative regulators did not change.

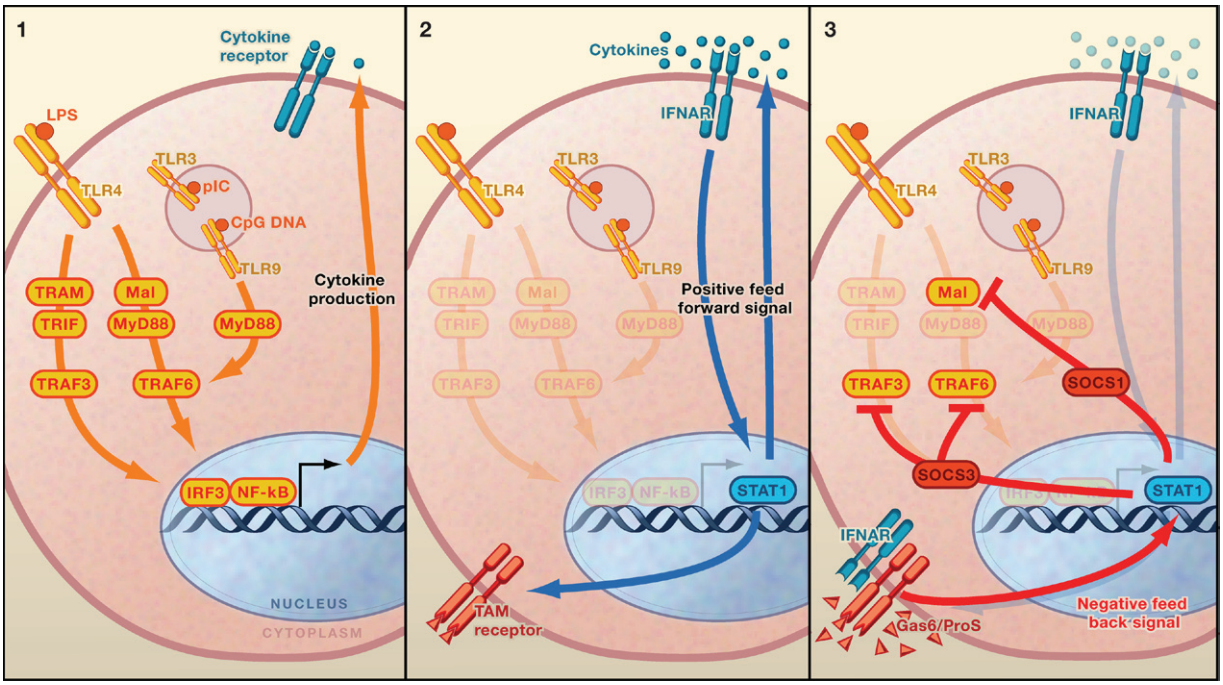

Figure 1. Inhibition of TLR Signaling by TAM Receptors

Rothlin et al. (2007) propose a tripartite model for TLR action. In the first stage (1), an initial response is triggered by TLRs via adaptor proteins that lead to activation of TRAF3 and TRAF6. In turn, TRAF3 and TRAF6 activate the transcription factors IRF3 and NF-κB, respectively, leading to production of cytokines, notably type I interferons, which promote host defense and inflammation. In the second stage (2), interferon signaling has a positive feedforward effect, promoting further interferon production via STAT1, thereby amplifying the response. However, in the third stage (3), interferons also induce inhibitory TAM receptors via STAT1 in a negative feedback effect. The TAM receptor ligands Gas6 and ProS also activate STAT1, which selectively induces production of SOCS1 and SOCS3. These proteins mediate the inhibition of TLR signaling by targeting Mal in the case of SOCS1 and TRAF3 and TRAF6 in the case of SOCS3. Together, this process allows the innate immune response to be launched but ultimately ensures that it is self-limiting and therefore not detrimental to the host.

Rothlin and colleagues then probed the mechanism of SOCS1 and SOCS3 induction by TAM receptors. The transcription factor STAT1 was shown to be essential for SOCS1 and SOCS3 induction, as was the type I interferon receptor (IFNAR1). The TAM family member Axl was found to associate with the R1 chain of IFNAR1. Therefore, a new pathway involving the ligands Gas6 and ProS had been uncovered, and it activates STAT1 via TAM receptors in a complex with R1-IFNAR1, leading to the induction of SOCS1 and SOCS3. SOCS3 likely prevents polyubiquitination of TRAF3 and TRAF6, key signaling proteins activated by TLR3, TLR4, and TLR9. The final part of the mechanism involves the induction of TAM receptors in response to TLR ligands. Intriguingly, this was also shown to depend on type I interferon signaling such that TLRs induce interferon-α, which activates STAT1 via IFNAR1, leading to induction of TAM receptors. The authors conclude that TAM receptors activate the terminal signal in what they term a tripartite inflammatory cycle. This cycle comprises the ignition of inflammation activated by TLRs, amplification of the signal by type I interferons (and other cytokines) in a positive feedforward mechanism, and finally TAM-mediated inhibition of TLR signaling in a negative feedback mechanism, which occurs via induction of SOCS1 and SOCS3 (Figure 1).

These events reveal the complexity of TLR regulation. Inflammation is initiated by TLR signaling, which involves the production of a range of cytokines and chemokines to elicit the effector mechanisms of host defense. The cytokines can also trigger amplification in the overall responses, including enhancing their own induction. The transcription factor STAT1 is particularly important here, as it is required for signaling by type I interferons. However, TAM receptors are also induced by cytokines and this induction (acting via STAT1) leads to increased expression of SOCS1 and SOCS3. SOCS1 targets the key TLR2 and TLR4 adaptor protein Mal (Mansell et al., 2006), whereas SOCS3 targets TRAF6 (used by all TLRs) and TRAF3 (used by TLR3 and TLR4) (Rothlin et al., 2007; Frobose et al., 2006). Therefore, between SOCS1 and SOCS3 there is the capacity to inhibit all TLRs.

The authors also conclude that the induction of SOCS1 by interferons can be almost wholly explained by TAM receptors. This prompts the first important question to arise from this study. What is the source of the TAM receptor ligands Gas6 and ProS? There is some evidence that they are autocrine factors, although this seems unlikely. The authors provide the interesting speculation that regulatory T cells, which are well-known

inhibitors of TLRs, might be a source. The second question is why TAM receptors do not activate a range of STAT1-dependent genes. Instead, the effects of TAM receptors appear to be only inhibitory. The authors speculate about alternative effects on STAT1, but these require testing. Perhaps the IFNAR1/TAM receptor complex signals differently from IFNAR receptor complexes that bind to interferons. Related to this possibility, future work may address in detail how Axl interacts with and regulates IFNAR1.

Given the importance of TLRs for the pathogenesis of autoimmune and inflammatory diseases, these findings will further stimulate efforts to modulate TLR activity. If the effects of the TAM receptor ligands Gas6 and ProS are specific to dendritic cells, these two factors might find use as anti-inflammatory agents. Furthermore, hyperactive TAM signaling might play a role in sepsis by blocking host defense responses. Consistent with this notion, elevated levels of Gas6 have been detected in patients with severe sepsis. An inhibitor of TAM receptors (such as a small molecule that blocks TAM receptor kinase activity) might potentiate immune responses in immunosuppressed patients. Such an inhibitor could also be used as a vaccine adjuvant as there is a pressing need to enhance the immunogenicity of candidate vaccine antigens.

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Sticklebacks and Humans Walk Hand in Fin to Lighter Skin

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In this issue, Miller et al. (2007) show that evolution makes repeated use of the same genes to produce light pigmentation in animals as divergent as stickleback fish and humans. This study indicates that analyzing parallel evolution at the genetic level could help to answer a number of outstanding questions in evolutionary genetics.

To what extent is evolution repeatable? One classic way to address this issue is to ask whether evolutionary change occurs in parallel, that is do the same traits evolve repeatedly in disparate taxa? Evidence of parallel evolution at the phenotypic level has come from many studies of experimental evolution in microbes (e.g., Travisano et al., 1995). These studies show overriding patterns of repeatable, predictable change; however, the details vary, muddying those patterns. Thus, it remains unclear how deep the parallel changes go. With the advent of the genomics era this question can now be asked at the genetic level for natural populations. Does evolution tinker with the same genes to produce parallel phenotypic change or are there many routes to the same phenotype?

In this issue, Miller and colleagues (2007) tackle this question by exploring genetic mechanisms underlying pigmentation differences between marine and freshwater species of threespine stickleback fish (*Gasterosteus* species complex). Sticklebacks are well known for their variation in color: from red and blue, to jet black, to translucent white. In the midst of large-scale