Immunology and Cell Biology (2008) 86, 299–300

www.nature.com/icb

NEWS AND COMMENTARY

Regulating inflammation

The ying and yang of NF-κB activation

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Immunology and Cell Biology (2008) **86,** 299–300; doi:10.1038/icb.2008.21; published online 8 April 2008

If you have memories of learning to ride a bicycle, you may remember that once you were moving, the real problem was mastering the ability to stop. This capacity to 'stop' presents a significant problem for regulation of the immune system. Indeed, from one perspective, chronic and persistent autoimmune conditions could be viewed as a failure to 'stop'. Given the complexity of the immune system, it would come as no surprise to discover that a myriad of intricate mechanisms exists to temper, and ultimately terminate, immune responses following their initiation. A recent series of articles in *Nature Immunology*[1,2] have further unraveled this complexity, revealing new insights into the molecular mechanisms by which the nuclear factor κB (NF-κB)-dependent gene A20 regulates immune-cell activation.

During inflammation, the transcription factor NF-κB integrates signals received by the T-cell receptor (TCR), Toll-like receptors (TLR) and cytokines, such as tumor-necrosis factor (TNF) and interleukin-1 (IL-1), to regulate the transcription of other proinflammatory genes (reviewed in Hayden and Ghosh[3]). These NF-κB-target genes are necessary for immune activation and the clearance of pathogens. In unstimulated cells, NF-κB proteins reside in the cytoplasm, retained by the inhibitory IκB molecules. Following activation, such as through the TCR or TNF receptor, distinct signaling cascades are activated that converge upon IκB kinase (IKK) complex. The trimeric IKK complex phophorylates IκBα, leading to its degradation by the proteosome and allowing NF-κB to translocate to the nucleus, where it activates target genes. Whereas on one hand, NF-κB is important for the activation of proinflammatory genes, it is also essential for the resolution of inflammation and protection from apoptosis. Indeed, unregulated activation of NF-κB *in vivo* is lethal[4] which, at the cellular level, is associated with unfettered immune activation and increased susceptibility to programmed cell death.[5] Emerging evidence indicates that this seemingly paradoxical function of NF-κB is mediated, at least in part, by its ability to coordinately induce expression of genes, such as A20 (otherwise known as TNFAIP3).

Over recent years, A20 has emerged as a major regulator of inflammation. The most dramatic demonstration of its importance in regulating immune function can be observed in A20-deficient mice that are born cachectic and died within 3 weeks of birth as a result of unchecked inflammation.[6] A20 was originally identified as a TNF-inducible gene product in human umbilical vein endothelial cells,[7] but was later shown to be expressed by various hemopoietic and non-hemopoietic-lineage cells. A20 is itself an NF-κB-target gene,[8,9] which functions to inhibit NF-κB activation at a level upstream of IκBα phosphorylation.[10,11] A20 then comprises a negative feedback loop, being required to terminate NF-κB signaling, preventing the otherwise deleterious consequences associated with uncontrolled NF-κB activation. This is consistent with the concept that A20 performs a necessary immunological 'stop' function. However, given that A20 is an immediate response gene,[7,9] one would expect that its expression would immediately shut down NF-κB signaling, allowing only on–off binary control of NF-κB activation.

In a very exciting new development, Coornaert *et al.*[1] have demonstrated the existence of a complex interaction between A20 and proximal signaling units of the TCR. Indeed, their study reveals a much more labile control system, which presumably can allow fine-tuning of nascent T-cell activation. Ligation of the TCR by major histocompatibility/peptide complexes activates NF-κB and this is dependent upon the recruitment and subsequent activation of a multimeric complex of which the protein MALT1 plays an important role.[12] Interestingly, MALT1 contains a caspase-like domain, however, to date no caspase activity for MALT1 has been identified. Of further interest, A20 is constitutively expressed in T cells,[1] thereby potentially acting as a permanent roadblock for TCR-dependent activation of NF-κB. Thus, for NF-κB activation to occur and T-cell activation to proceed, A20 must be removed, an event that Coornaert and colleagues show is achieved by MALT1-dependent degradation (Figure 1a). In their study, they demonstrate that A20 is specifically recruited to the TCR-signaling complex, but only after cellular activation and is then proteolytically degraded by the caspase activity of MALT1. Catalytic degradation of A20 prevented its ability to inhibit NF-κB-activation and allowed T-cell activation to occur as indicated by the induction of IL-2 (Figure 1a). In a feedback circuit, activation of NF-κB would be expected to initiate the *de novo* synthesis of more A20, which will then function to block TCR-dependent NF-κB activation unless A20 is again subjected to MALT1-dependent degradation. This collectively reveals the dynamic nature of TCR-mediated NF-κB signal transduction.

Studies on the role of A20 indicate that it is capable of preventing NF-κB activation by multiple stimuli at a level upstream of IκBα phosphorylation. Given the convergence of multiple NF-κB-activating stimuli at the IKK complex,[3] evidence that A20 could interact with the IKK NEMO sub-unit[13] hinted at a possible means by which A20 could achieve this broad inhibitory function. However the exact mechanism by which A20 interfered with NF-κB activation remained elusive. An important study by Dixit's group, the original

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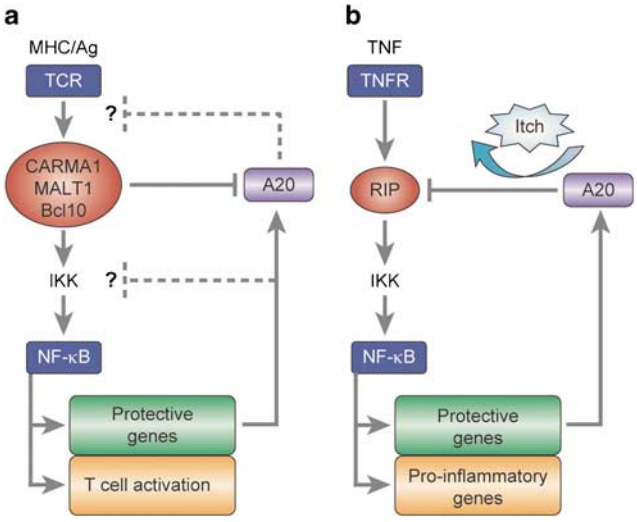

Figure 1 Modulation of nuclear factor κB (NF-κB) signaling through A20. (**a**) Ligation of the T-cell receptor (TCR) activates NF-κB resulting in the transcription of proinflammatory genes, such as those required for T-cell activation, but also the anti-inflammatory gene A20. A20 can halt NF-κB activation, possibly by inactivating NEMO at the level of IκB kinase (IKK) or other targets proximal to TCR signaling. MALT1 allows NF-κB activation to proceed through activation of IKK and by inactivating A20 through proteolysis. (**b**) A20 can inhibit TNF signaling at the level of receptor-interacting protein, a step that requires the activity of Itch and TAX1BP1. Collectively these studies reveal the dynamic and complex nature of NF-κB signal transduction mediated by A20.

team that identified A20 as a TNF-inducible gene product,[7] showed that A20 could bind the receptor-interacting protein (RIP) and target it for proteolytic degradation.[11] The kinase activity of RIP is essential for TNF-mediated activation of NF-κB, thus providing an important mechanistic insight into how A20 attenuates signaling through the TNF receptor. However, the problem of the binary nature by which A20 regulates RIP-dependent activation of NF-κB remains. In a second installment to our knowledge of how A20 functions, Shembade *et al.*[2] now show that once recruited to RIP, A20 is subjected to regulation by a complex consisting of the molecules Itch and TAX1BP1 (Figure 1b). In the absence of Itch, A20 cannot be recruited to the RIP complex and NF-κB activation cannot be terminated. Consistent with this concept, they further demonstrate that Itch-deficient mice exhibit a phenotype reminiscent of $A20^{-/-}$ mice; that is, $Itch^{-/-}$ mice exhibit T cell-intrinsic enhanced TNF-mediated NF-κB activation and premature death. Presumably, Itch or TAXBP1 can act as modulators of TNF signaling by their ability to control A20 subcellular localization and activation. Collectively, these studies reveal the dynamic and complex nature of NF-κB signal transduction mediated by A20.

With regards to these two new studies, the earlier result by Dixit's team demonstrating that A20 regulates TNF signalling by degrading RIP raises an important question. How does A20 prevent NF-κB activation triggered through the TCR, which ostensibly signals independently of RIP? Though the study by Coornaert does not identify how A20 might block signaling through the TCR, their study raises the possibility that A20 targets degradation of one or more of the molecules associated with MALT1, such as Bcl-10 or CARMA1/CARD11. Indeed, one inference might be that A20 can interact with multiple proximal signaling units specific for each NF-κB-activating pathway. Though TCR-specific A20 targets have not as yet been identified, the elucidation of such would constitute a fruitful target area for future studies.

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