CHAPTER 4

TRAF3 and Its Biological Function

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Abstract

Tumor necrosis factor receptor associated factor 3 (TRAF3) is one of the most enigmatic members in the TRAF family that consists of six members, TRAF1 to 6. Despite its similarities with other TRAFs in terms of structure and protein-protein association, overexpression of TRAF3 does not induce activation of the commonly known TRAF-inducible signaling pathways, namely NF-κB and JNK. This lack of a simple functional assay in combination with the mysterious early lethality of the TRAF3-deficient mice has made the study of the biological function of TRAF3 challenging for almost ten years. Excitingly, TRAF3 has been identified recently to perform two seemingly distinct roles. Namely, TRAF3 functions as a negative regulator of the NF-κB pathway and separately, as a positive regulator of type I IFN production, placing itself as a critical regulator of both innate and adaptive immune responses.

Introduction

Beginning in the 1990s, many studies were emerging reporting the discovery of a diverse family of surface receptors which have collectively been come to known as the tumor necrosis factor superfamily of receptors (TNFRs).[1] Currently, more than 29 members have been identified. These receptors are grouped together based on the similarity of their extracellular domains which contain cysteine-rich regions. Each of these TNFRs plays a significant and unique role in fundamental biological processes and, importantly, deregulation of signaling pathways downstream of these TNFRs are believed to be causative factors in many immune and inflammatory diseases.[2] Consequently, the scientific and medical communities possess a tremendous interest in the characterization of signaling mediators downstream of these receptors in the hope of identifying therapeutic targets for the treatment of related diseases.

In 1994, TRAF1 and TRAF2 were the first molecules identified as associating factors to TNFR II.[3] Accordingly, these molecules were given the name tumor necrosis factor receptor associated factors (TRAFs). At a similar time, TRAF3 was identified through its association with the cytoplasmic tails of CD40 and the Epstein-Barr virus latent membrane protein (LMP-1).[4-6] Given the fact that TRAF proteins shared significant sequence homology and the emerging studies showing TRAFs association with multiple TNFRs, researchers speculated that members of the TNFR superfamily may initiate their specific signal transduction cascades by recruitment of specific TRAF proteins. To date, six TRAFs have been identified and are grouped as a family of intracellular adaptors which transmit signals downstream of most if not all of the TNFRs as well as other non-TNF receptors such as the toll-like receptors. As such, TRAF proteins mediate a plethora of biological functions; the most well studied involving the initiation of innate and adaptive immune responses against pathogen infections.

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Characterization of TRAF3

TRAF3 is a ubiquitously expressed protein, suggesting that it may perform significant physiological and cellular functions in multiple organs. TRAF3 expression has been observed in many murine tissues, including brain, heart, lung, liver, spleen and thymus.[6] It is also expressed in several human cell types including myeloid progenitor cells, monocytes, and plasma cells.[7]

The TRAF3 protein is evolutionarily conserved between human and mouse, with 96% of their amino acid sequence being identical.[6] The human TRAF3 protein is composed of 568 amino acids with a molecular weight of approximately 64kDa. Similar to all the other TRAF members, TRAF3 possesses a signature TRAF domain at the carboxyl terminus. At the N-terminus, TRAF3 contains a typical C3HC4 RING finger domain, followed by five zinc-binding fingers, and an isoleucine zipper. While the TRAF domain has been shown to be important for binding to the cytoplasmic domain of tumor necrosis factor receptor (TNFR) family members, intracellular signaling mediators, and for forming homo- or hetero-dimers with other TRAFs, the function of the other TRAF3 domains has yet to be characterized.[8-11]

Structure-Function Study of TRAF3

Although TRAF3 has similar secondary structures as other TRAFs, over-expression of TRAF3, in contrast to TRAFs 2, 5 and 6, fails to activate the JNK or NF-κB pathways.[12-16] To understand the structural basis for the functional differences between TRAF3 and its family members, various domains of TRAF3 were replaced with domains from TRAF5, its closest TRAF member in terms of amino acid identity. Results indicated that the first zinc finger and 10 residues of the second zinc finger of TRAF5 are sufficient to convert TRAF3 into an activator of both JNK and NF-κB pathways.[17] This suggests that the zinc fingers of TRAF3 contribute to its inability to activate both JNK and NF-κB. Interestingly, the cellular localization of TRAF3 seems to differentiate it from the other TRAF family members as TRAF3, unlike TRAF2, 5 and 6, is not preferentially localized to the insoluble cell pellet fraction.[18] This may partly explain the differences in pathway activation potential exhibited by TRAF3 in comparison to other TRAFs. In support of this, myristoylation of TRAF3, which forces TRAF3 to the insoluble membrane fraction, converts TRAF3 into an activator of the JNK pathway.[18]

TRAF3 Association with Surface Membrane Receptors

Following the initial biochemical identification of TRAF3 as a CD40 associating factor, a tremendous amount of effort was put forth to uncover its role in CD40 signaling and biology. However, this endeavor was complicated by the observation that multiple TRAFs, including TRAF2, 3, 5, and 6, can bind to CD40 and that TRAF2 and 3 even bind to an overlapping region. Nevertheless, initial studies indicated an inhibitory role for TRAF3 in CD40 biology as overexpression of this protein inhibits CD40-induced CD23 expression and antibody secretion in B cells.[6,19] However, overexpression of a CD40 mutant which abolishes TRAF3 but not TRAF2 binding, had no effect on CD40-mediated NF-κB and JNK activation which suggests a neutral role for TRAF3 in CD40 signaling transduction.[20] In agreement with the latter finding, CD40-induction of CD23 expression and NF-κB activity were normal in a TRAF3-deficient cell line and antibody secretion and JNK activity were only slightly increased.[21] Consequently, it remained unclear if TRAF3 plays a significant role in CD40 signaling.

The role of TRAF3 in LMP-1 signaling has also been extensively investigated. LMP-1 is a transforming protein from Epstein Barr virus which mimics the signaling characteristics of constitutively active CD40. Like CD40, LMP-1 can associate with TRAF 2 and 3 and activate the NF-κB and JNK pathways.[22,23] Analogous to CD40, LMP-1 induces expression of B cell markers ICAM-1, LFA and CD23.[24] In contrast to its unidentified role in CD40 signaling, TRAF3 appears to function as an important mediator of LMP-1 signal transduction. In one study, using a TRAF3-deficient B cell line that stably expresses LMP-1, results indicated that TRAF3 served a positive role in LMP-1 activation of NF-κB and JNK.[21] This observation may be explained by the innate differences between these two receptors. For example, LMP-1 appears to have a higher affinity for TRAF3 than CD40 and unlike CD40, LMP-1 does not induce the degradation of TRAF3.[25,26] Still, how these differences actually contribute to the differential roles of TRAF3 in CD40 and LMP-1 signal transduction remains unclear.

Following the identification of TRAF3 association with CD40 and LMP-1, an increasing number of TNF receptors have been shown to bind to TRAF3.[10] All these receptors share a domain called the TRAF interacting motif (TIM). This TIM sequence can vary between receptor to receptor, but can be generally described as (P/S/A/T)X(Q/E)E which is found in CD40, CD30, HVEM, OX40, p75NGFR, and RANK.[12,27-29] Intriguingly, for some receptors, TRAF2 and 3 seem to be able to bind to the same TIM. For instance, both TRAF2 and 3 bind to PVQET on CD40.[30] This suggests that TRAF3 may compete with TRAF2 for binding to the receptor and/or that TRAF2 and TRAF3 may form a signalosome when receptors are oligomerized. Indeed, TRAF2 and 3 form heterodimers though the importance of this partnership remains to be determined.[31] In addition, crystal structures of the TRAF domain of TRAF2 and 3 and a CD40 peptide encompassing the TRAF2/3 binding motif showed that CD40 assumed different conformations depending on which of these two TRAFs it binds.[32,33]This provides a possible scenario where CD40 may elicit unique and specific signaling outcomes depending on the TRAF complex bound to its cytoplasmic tail.

TRAF3 Interacting Molecules

In addition to characterizing TRAF3 association with surface receptors, extensive effort was focused on identifying TRAF3 associating molecules in an attempt to uncover its function. This approach yielded a number of TRAF3-associating molecules including Act 1, ASK1, c-src, MIP-T3, NIK, p62 nucleoporin, p85 subunit of PI-3K, $p40^{phox}$, RIP1, RIP4, TANK, T3JAM, TNAP and TTRAP.[15,34-46] Among all these molecules, many of them can bind to the other TRAF members as well, whereas MIP-T3, p62 nucleoporin, and T3JAM appear to specifically bind to TRAF3.[35,36,40] Further studies are required to establish the physiological roles of these associated proteins in TRAF-mediated biological events.

Phenotype of TRAF3-Deficient Mice

Besides using a biochemical approach to study the function of TRAF3, a genetic approach was also employed. TRAF3-deficient mice were generated in 1996. Despite a relatively normal gestation period, Traf3 knockout mice rapidly degenerated after birth with symptoms including stunted growth and progressive hypoglycemia, hypercortisolemia, and leucopenia resulting in a premature death within two weeks of age.[48] Despite numerous efforts, the instigating factor in this perinatal lethality remained undetermined for many years.

Because TRAF3 was identified as a CD40-associating molecule, the role of TRAF3 in the CD40 pathway was assessed in TRAF3 null cells.[48] In vitro stimulation of *Traf3*$^{-/-}$ B cells with anti-IgM and CD40L showed no difference in proliferation compared to wild-type cells. Furthermore, *Traf3*$^{-/-}$ B cells showed no defect in upregulating B7.1 and CD23 upon CD40 ligation. Therefore, TRAF3 is not required for CD40-induced B cell proliferation and activation. However, TRAF3 was involved in generating an immune response to T-dependent antigen. Mice reconstituted with *Traf3*$^{-/-}$ fetal liver cells could not mount a proper immune response to a T-dependent antigen. In addition, in vivo primed *Traf3*$^{-/-}$ T cells were defective in proliferative responses to antigen presentation. It remains to be determined whether this defect is in result of problems with *Traf3*$^{-/-}$ antigen presenting cells or in T helper cell functions. Due to the promiscuity of TRAF3 in binding to at least twenty TNF receptors and the ubiquitous expression of TRAF3, generation of cell-type specific or tissue-specific disruption of the TRAF3 gene is necessary to tease out the role of TRAF3 in different cell types and organs.

Breakthrough in Identification of TRAF3 Function: The Noncanonical NF-κB Pathway

As mentioned above, unraveling the mystery of TRAF3 function had proven difficult due to the early post-natal lethality of *Traf3*$^{-/-}$ mice and the failure of traditional biochemical studies to establish a link between TRAF3 and known signal transduction pathways. Five years after the targeted disruption of TRAF3, however, studies began to emerge about a second, evolutionary conserved NF-κB activation pathway, and pointed in a new direction for the study of TRAF3 function.

In brief review, the NF-κB family of transcription factors plays pivotal roles in the propagation of innate and adaptive immune responses through the activation of multiple gene targets including

those involved in cell growth, survival, apoptosis, and inflammation.[49,50] Five NF-κB family members exist in mammals: NF-κB1 (encoding p105 which is constitutively processed to p50), RelA (p65), cRel, NF-κB2 (encoding p100 which is processed to p52), and Rel B. Under normal conditions, inactive Rel dimers are retained in the cytoplasm through interaction with one of a family of inhibitory molecules, termed inhibitors of κB (IκBs).[51] Signal-dependent phosphorylation of IκBs on key serine residues results in IκB degradation and the translocation of Rel dimers capable of binding DNA in the nucleus.[52]

Classical or canonical NF-κB activation requires the IκB kinase (IKK) complex which consists of two catalytic subunits (IKKα and IKKβ) and one regulatory subunit (NEMO/IKKα). IKK activation results in the degradation of IκBα and -β which release p50:RelA and p50:cRel dimers.[53,54] Activation of the 'alternative' or noncanonical pathway involves activation of NF-κB inducing kinase (NIK) which associates with two molecules of IKKα.[55,56] Together, NIK and IKKα, bind to the C-terminal portion of p100 (also termed IκBδ) leading to the processing of p100 to p52 and the release of p52:RelB dimers.[57-59] Another important distinction between these two NF-κB activation pathways involves the kinetics/pattern of activation and the requirement for new protein synthesis. Here, canonical NF-κB activation occurs within minutes post-stimulation and does not require new protein synthesis. In addition, canonical NF-κB activation leads to the induction of IκBs which results in strong negative feedback. As a consequence, canonical NF-κB activation is characterized by an oscillatory function with decreasing amplitude over time.[60] In contrast, activation of the noncanonical NF-κB pathway requires several hours, new protein synthesis and does not decrease in strength over time.[61] While targeted disruption of Rel family members has identified overlapping functions in cellular proliferation and survival, they have also identified specific and unique biological roles for individual Rel proteins.[53] Importantly, disruption of signaling components of the noncanonical NF-κB pathway present highly similar phenotypes characterized by severely disorganized splenic and lymph node architecture, reduced B-cell numbers in the bone marrow and periphery, and defective T-dependent and independent immunologic responses.[62,63] At the same time, mice deficient in LTβR, CD40, or BAFFR, all of which strongly bind TRAF3 and activate the noncanonical NF-κB pathway, present with similar phenotypes suggesting a connection between TRAF3 receptor binding and noncanonical NF-κB activation.[59,61,64-67]

The first study that clearly establishes a link between TRAF3 and noncanonical NF-κB activation was performed by Liao et al.[68] Here, under overexpression in 293T cells, the authors showed via coimmunoprecipitation, a strong interaction between NIK and TRAF3. The authors further demonstrated that overexpression of TRAF3 resulted in a marked decrease in NIK levels and that siRNA-mediated suppression of endogenous TRAF3 resulted in accumulation of NIK and increased processing of p100 to p52. Finally, the authors showed that inhibition of the proteasome resulted in the accumulation of ubiquitinated NIK, and strikingly, that a NIK mutant lacking a short sequence which mediates TRAF3 binding, was protected from ubiquitination in this assay. This study therefore suggests that TRAF3 plays a crucial role in the suppression of NIK activity. Importantly, the authors were unable to see TRAF3 mediated ubiquitination of NIK in a standard 293T cell assay using an exogenous tagged form of ubiquitin, which strongly suggests that while TRAF3 is necessary for the negative regulation of NIK, it is also not sufficient. While the Liao et. al. study was compelling, the history of TRAF3 study suggested that the field should wait for a corroborative study before embarking on this new path of examination of TRAF3 biology. Conveniently, this condition was soon met by Hauer et. al. in a study showing that overexpression of any TNFR family member capable of binding TRAF3 led to nuclear accumulation of p52 and that dual overexpression of TRAF3 prevented this event.[69] Together, these studies strongly suggest that TRAF3 negatively regulates the processing of p100 to p52 through suppression of NIK. How might this occur? One possibility involves the observation that ligation of TRAF3-binding TNFR receptors results in TRAF3 degradation.[70] This suggests a simple model of noncanonical NF-κB activation wherein p100 processing is constitutively inhibited by TRAF3 mediated degradation of NIK. Upon appropriate receptor ligation, TRAF3 is recruited and degraded allowing for accumulation of NIK and activation of IKKα thus explaining the delayed kinetics and protein synthesis-dependent nature of noncanonical NF-κB activation. Can it be this simple? Probably not. First, it was recently reported that loss of TRAF2 also results in constitutive activation of

the noncanonical NF-κB pathway indicating that TRAF2 and TRAF3 (and possibly additional molecules) cooperate in the negative regulation of NIK.[71] Second, TRAF proteins have only been shown to have ubiquitin ligase activity for Lys-63 linkages which are not associated with protein degradation but rather the promotion of complexes and signal activation (similar to the role of tyrosine phosphorylation in signal transduction).[72] As such, it remains to be seen whether or not TRAF3 contributes to the negative regulation of NIK through Lys-63 or Lys-48 (proteasome targeting) ubiquitin linkages or simply as an adaptor molecule which recruits enzymatic components that regulate NIK stability. In depth analysis of the domains of TRAF2 and TRAF3 required for the negative regulation of NIK will be required to elucidate the complex mechanism of noncanonical NF-κB activation.

Previous genetic studies involving constitutive activation of the canonical (by deletion of IκBα) and noncanonical (by deletion of the p100 C-terminus) NF-κB activation pathways have show the critical importance of proper regulation of NF-κB activity (Fig. 1).[73,74] In consideration of this and these recent biochemical studies indicating that TRAF3 functions as a critical negative regulator of noncanonical NF-κB activity, one wonders how this may relate to the cause of the TRAF3-null phenotype. Indeed, it was recently reported that the TRAF3 null phenotype can be rescued by the compound deletion of the p100 gene.[75] So, 10 years after its discovery, the scientific community now has a much better understanding of why so many TNFR family members critical to the propagation of adaptive immune response recruit the enigmatic adaptor molecule, TRAF3.

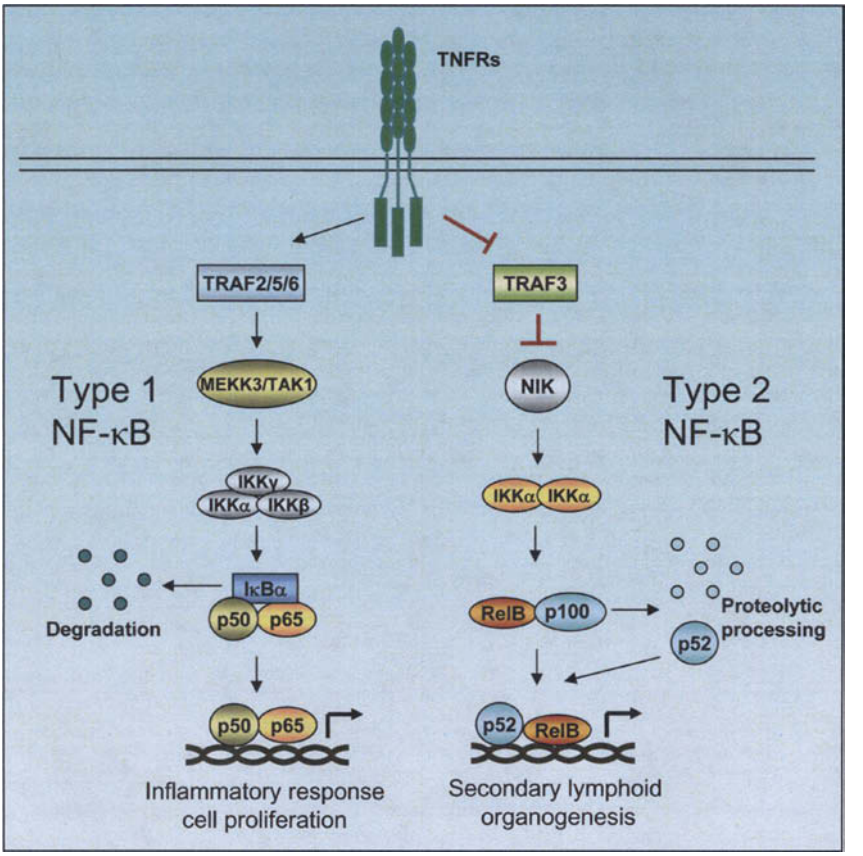

Figure 1. A schematic illustrating how TRAF3 may be involved in the activation of the noncanonical NF-κB pathway by TNFR family members such as BAFFR, CD40, and LTβR.

TRAF3 in Innate Immunity

At this point, the function of this mysterious TRAF family member might seem straightforward. TRAF3 acts as a powerful negative regulator of the noncanonical NF-κB pathway and this function is somehow inhibited through direct interaction with certain members of the TNFR superfamily, such as CD40 and BAFFR. However, two different lines of evidence began to emerge that hinted at another highly unexpected role for this molecule.

The type I IFN family of cytokines, composed of multiple IFNα's, IFNβ, and a few other subtypes, make up the most vital component of our innate immune response against viral infection. In addition, they play a major role in enhancing adaptive immunity and have been closely linked to autoimmune diseases such as System Lupus Erythematosus.[31,76] Thus, the mechanisms by which type I IFNs are produced by both leukocytes and stromal cells following viral infection or Toll-like receptor (TLR) ligation has been a major focus of attention in recent years. In addition to bacterial products such as LPS or flagellin, certain TLRs that localize to endosomes can recognize viral products such as dsRNA, ssRNA, and unmethylated CpG motifs (CpG) in DNA. In macrophages and plasmacytoid dendritic cells (pDCs), recognition of these products by TLRs 3, 7, and 9, respectively, results in the potent induction of type I IFNs.[77]

TLRs are a family of transmembrane receptors that represent an evolutionarily conserved recognition system for pathogen associated molecular patterns (PAMPs) found in microbial pathogens. Like the TNFR superfamily, the TLR family can potently activate NF-κB; however, TLRs can also induce antiviral responses through a family of cytokines called type I interferons (IFNs). Also like TNFR family members, TLRs require a member of the TRAF family to activate NF-κB, specifically TRAF6. Rather than directly binding the cytoplasmic receptor tail, as is the case in TNFR recruitment of TRAFs, TRAF6 is activated by TLRs through a signaling complex involving MyD88, IRAK4, and IRAK1. TLR3 is unlike most other TLRs by virtue of its potent activation of the antiviral response in macrophages and its predominant utilization of the adapter TRIF rather than MyD88.[78] The additional recent finding that TRAF6 is not required for TLR3 signaling left open the possibility that another TRAF family member may take its place in the TRIF-dependent pathway.[79]

Not long after its discovery, TRAF3 was used as bait in a yeast-two hybrid screen to identify novel interacting molecules. One of the strongest TRAF3 interacting molecules by yeast two hybrid screen was an adapter protein with unknown function later termed TANK for TRAF-associated NF-κB activator.[34,45] TANK was subsequently used in a yeast-two hybrid screen to identify an IKK-related molecule coined TBK1 for TANK-binding kinase1.[80,81] While TBK1 is homologous to IKKα and IKKβ, TBK1 is not involved in NF-κB activation. Instead, TBK1 and its close relative IKKε were later shown to be critical kinases of IRF3, one of the major transcription factors for type I IFNs.[82] For example, *Tbk1*$^{-/-}$ cells are defective in the antiviral response to TLR activation.[83,84] Thus, several lines of evidence suggested the possibility that TRAF3 may be involved in the regulation of antiviral responses.

When TRAF3-deficient macrophages were stimulated with the TLR3 ligand, polyI:C, the surprising possibility was confirmed. *Traf3*$^{-/-}$ macrophages treated with a synthetic form of dsRNA produced far less type I IFNs than their wild-type counterparts. Further study traced this phenotype to a failure of TRAF3-deficient macrophages to activate the type I IFN transcription factor IRF3. In contrast, TRAF3 was not required for activation of NF-κB by any of the TLRs tested. The fact that TRAF3 could also associate with both TRIF and TBK1 in coimmunoprecipitation studies suggested that TRAF3 may be linking TRIF to downstream IRF3 phosphorylation by TBK1.[75]

Plasmacytoid dendritic cells, the most potent known producers of type I IFNs, have demonstrated the ability to recognize different viruses through distinct TLR receptors based on the structure of the viral genome.[85,86] For instance, TLR7 is required for the recognition of the ssRNA viruses such as influenza and VSV, whereas TLR9 is required for recognition of DNA viruses including HSV-1, HSV-2, and MCMV.[87-89] This recognition event, which can be mimicked by synthetic TLR7 and TLR9 ligands, R848 and CpG, results in the secretion of high levels IFNα by the pDCs in a manner that depends on both MyD88 and IRAK1.[78] Because TLRs 7 and 9 utilize MyD88 rather than TRIF, it was an additional surprise when it was found that *Traf3*$^{-/-}$ pDCs are

also greatly defective in the antiviral response to ligation of TLRs 7 and 9. However, further study suggested that TRAF3 may actually interact with IRAK1 to activate the transcription factor for IFNα, IRF7.[75] Thus two distinct pathways appeared to converge on TRAF3 to induce a specific antiviral response.

In contrast to pDCs, nonimmune cells do not appear to recognize viral infection via TLRs or other known surface receptors. Instead, cytoplasmic protein receptors are thought to directly bind viral components such as dsRNA and subsequently activate an appropriate cellular response, including the induction of type I IFNs. Recently, RIG-I and MDA5 (Helicard) have been implicated as potential receptors for the detection of intracellular viral infection in nonimmune cells such as murine embryonic fibroblasts (MEFs).[78] Interestingly, Cardif, a critical adapter for signaling by Helicard and RIG-I, contains TRAF binding motifs (TBMs) similar to those found in the CD40 receptor.[90-93] Thus, it is not too surprising that TRAF3-deficient MEFs failed to induce type I IFNs following direct viral infection. In fact, *Traf3*$^{-/-}$ MEFs were several fold more susceptible to viral infection.[75] Although TRAF3 was previously only thought to be involved in adaptive immunity due to its association with CD40, BAFF, and LTβ receptors, it now seems apparent that this molecule plays a major role in innate immunity as well (Fig. 2).

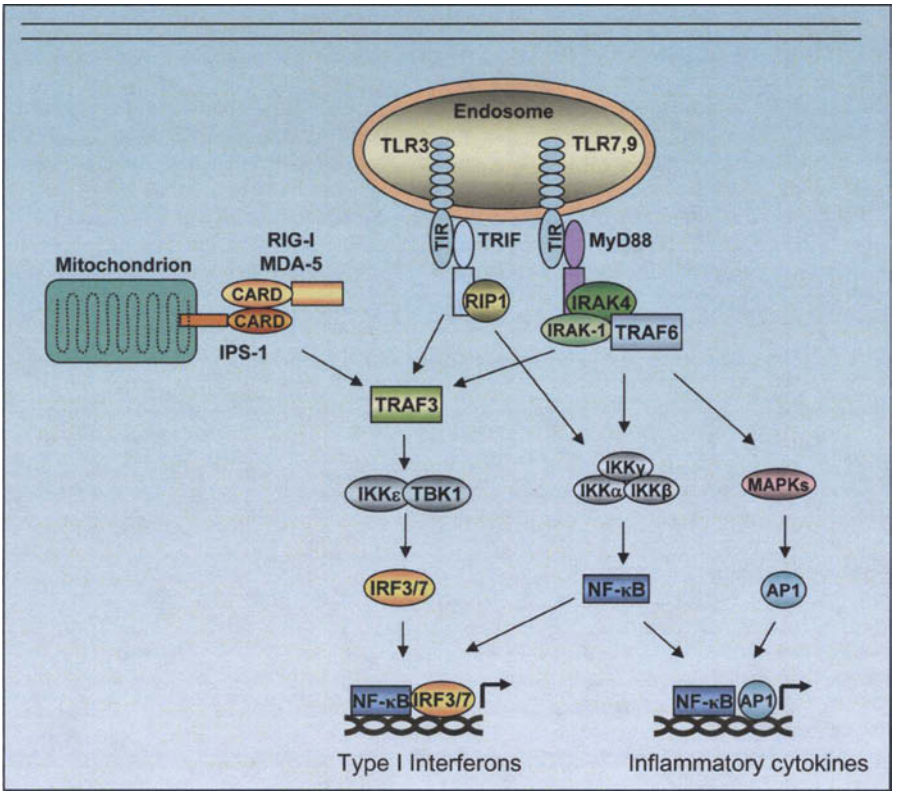

Figure 2. A schematic illustrating how TRAF3 may be involved in the activation of type I interferons by TLR-dependent and TLR-independent viral recognition pathways.

Concluding Remarks

How can TRAF3 simultaneously control two seemingly unrelated pathways, acting as a negative regulator in one and a positive regulator in another? A complete mechanistic understanding of how TRAFs function has remained elusive. TRAF6 is thought to activate NF-κB through an auto-ubiquitination event. The RING finger domain of TRAF6 acts as an E3 ligase for itself, resulting in the polyubiquitination of TRAF6. These ubiquitin chains then recruit a complex including TAK1, TAB1 and TAB2, which then results in the phosphorylation and activation of the IKK complex.[94] When proposing a model for TRAF3 function, one cannot ignore the striking homology between the TRAF3 and TRAF6 pathways. TAK1 and NIK, both MAP kinase kinase kinase family members, are thought to phosphorylate homologous residues on an activation loop, or "T-loop," of IKKβ or IKKα, respectively.[95] Interestingly, this same activation loop is present in both TBK1 and IKKε and required for their ability to activate IRF3. Thus, it would appear likely that similar mechanisms are governing TRAF6-mediated activation of the IKK family and TRAF3-mediated activation of TBK1/IKKε.

As mentioned above, biochemical studies have suggested that TRAF3 suppresses NF-κB by constantly mediating the degradation NIK.[68] Presumably, TRAF3 acts as an E3 ubiquitin ligase for NIK through its N-terminal RING finger domain. However, this has not yet been formerly proven. Is the same E3 ligase activity of TRAF3 involved in both NIK degradation and regulation of IRF transcription factors? Like other TRAFs, TRAF3 is composed of multiple domains capable of mediating numerous protein-protein interactions, including zinc fingers, an isoleucine zipper, and a common TRAF domain. Extensive structure function studies may one day reveal the relative contributions of these TRAF3 domains to its multiple distinct functions.

The recent progress toward understanding the functional role of TRAF3 now creates a more complete picture of the specificity involved in signaling by TNFR and TLR family members. While TRAF family members have homologous structures, the early lethality caused by loss of TRAF6, TRAF3, and TRAF2 expression is testament to their nonredundant and distinct roles. A detailed mechanistic understanding of how TRAFs are activated and translate that activation event to downstream pathways may therefore provide researchers with novel specific targets for therapeutic manipulation of numerous biological processes.

Although in vitro studies have implicated a role for TRAF3 in both adaptive immunity and innate antiviral responses, future in vivo functional analysis through the use of tissue-specific genetic disruption of the *traf3* locus will likely provide a more complete assessment for the potential of therapeutically targeting TRAF3-related pathways. Interestingly, Epstein Barr virus may have already discovered this potential as evidenced by the EBV-encoded transmembrane protein LMP-1, which specifically targets and binds TRAF3.[22,23] This sequestration of TRAF3 may serve the dual purpose of preventing antiviral responses resulting from the EBV infection in addition to simultaneously triggering constitutive noncanonical NF-κB activation, thereby extending the lifespan of EBV-infected B cells. Although this has yet to be demonstrated, it is likely that loss of TRAF3 function in B cells would result in the survival of autoantibody-producing B cells through this constitutive noncanonical NF-κB activity. On the other hand, TRAF3 is the only molecule known to be generally required for type I interferon production following both TLR ligation and viral infection in macrophages, pDCs, as well as fibroblasts.[75] The strong correlation between excessive type I interferon production, enhanced survival of autoantibody-producing B cells and autoimmune diseases such as Systemic Lupus Erythematosus (SLE) may place TRAF3 in the rare and delicate position of both suppressor and enhancer of autoimmune diseases. Thus, our current understanding of the biological importance of TRAF3 in both physiological and pathophysiological processes may just be the tip of the iceberg.

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