TRAF3: uncovering the real but restricted role in human

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In this issue of *Immunity*, Pérez de Diego et al. (2010) report a primary human immune deficiency involving a TRAF protein, and show that human immunity to the virus HSV-1 is critically dependent on TRAF3.

Tumor necrosis factor (TNF) receptor associated factors (TRAFs) are multi-functional intracellular proteins that recruit and activate protein kinases in immune signaling pathways. The function of many TRAF proteins has been particularly well established in TNF receptor family signaling in B and T lymphocytes, and in pattern recognition receptor (PRR) signaling in antigen presenting cells, mainly through the use of gene deleted mice. For example, TRAF6 has been shown to be critical for signaling by two PRR families, namely the Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs), where it controls activation of the transcription factor NF-κB, leading to the production of proinflammatory cytokines. However the function of TRAF3 remained enigmatic until 2006 since *Traf3*$^{-/-}$ mice die shortly after birth (Xu et al., 1996). In that year, two studies used *Traf3*$^{-/-}$ myeloid cells to show that TRAF3 was essential for induction of type I interferons (IFNs) in response to all the murine anti-viral nucleic acid sensing TLRs (TLR3, 7, 9), and also for TLR-independent type I IFN induction, whereas in contrast to TRAF6, there was no positive role for TRAF3 in NF-κB activation (Oganesyan et al., 2006; Häcker et al., 2006). For TLRs, TRAF3 acted

downstream of the TLR signaling proteins TRIF (for TLR3) and MyD88 and IRAK-4 (for TLR7 and 9), whereas TRAF3 acted in the RLR pathway downstream of the adaptor MAVS (Saha et al., 2006). Murine TRAF3 was also shown to negatively regulate the non-canonical NF-κB pathway in B cells, which involves processing of NF-κB p100 into NF-κB p52, and indeed constitutive activation of this pathway explained the postnatal lethality seen in *Traf3*$^{-/-}$ mice (He et al., 2006). Thus, in mice, using *Traf3*$^{-/-}$ cells, TRAF3 was assigned a positive role in type I IFN induction by PRRs, and a negative regulatory role in B cells. However, the role of TRAF3 in host defense *in vivo* and in immunity to infection was untested due to the lack of viable *Traf3*$^{-/-}$ mice. Furthermore, nothing was known about the role of TRAF3 (or indeed any TRAF) in human host defense.

In this issue of *Immunity*, Pérez de Diego et al. (2010) now provide insight into the immunological role of TRAF3 in host defense *in vivo*. Moreover, they do so in the human system, because they describe TRAF3 deficiency as a genetic susceptibility to herpes simplex virus-1 (HSV-1) encephalitis (HSE), a severe infection of the human central nervous system (CNS). Not only does this study provide real evidence for a role for TRAF3 in human immune responses, it also represents a demonstration of a primary immune deficiency involving a TRAF protein. Evidence is presented for an autosomal dominant TRAF3 deficiency in a young adult with a history of HSE in childhood, in that a single point mutation (R118W) of one *TRAF3* allele led to a loss-of-function, dominant negative phenotype, leading to greatly reduced expression of TRAF3 protein.

This TRAF3 study comes on the back of previous studies from the Casanova group where they have examined the clinical genetics of infectious diseases, and identified primary immune deficiencies that have allowed an important comparison

between the role of TLRs and their signaling components in experimental infections in animal models and in humans *in natura* (Quintana-Murci et al. 2007). Apart from the obvious importance of working in humans, these studies in a natural ecosystem governed by natural selection have the advantage that they dissect natural, and not experimentally-induced phenotypes relating to infection. They also allow the testing of paradigms developed in murine models. One such paradigm, that TLRs are critical in the host response to a broad range of infections, has been questioned by these studies (Quintana-Murci et al. 2007). This is because patients with deficiencies in TLRs or their signaling components seem susceptible to a much narrower range of infections that would be predicted by murine models, even though cells derived from such patients show the expected impaired responses to multiple pathogens. Thus IRAK-4- or MyD88-deficient patients suffer from pyogenic bacterial infections (such as pneumococcal disease) but seem to have normal resistance to all other microbes (Quintana-Murci et al. 2007; von Bernuth et al. 2008), whereas TLR3-deficient patients, in an identical phenotype to that reported here for TRAF3 deficiency, show a selective predisposition to HSE (Zhang et al. 2007).

The functional defect in TRAF3 observed here was due to reduced, but not completely depleted, amounts of TRAF3 protein in cells, because the mutant R118W protein exerted a destabilizing effect on normal TRAF3 protein expressed from the other allele. Evidence suggests that the R118W protein destabilizes TRAF3 trimer formation, which would explain the loss-of-function because activation of TRAFs requires their oligomerization.

The observation that TRAF3 deficiency in humans causes HSE specifically was not predicted by any of the murine work to date. The availability of cells expressing the TRAF3 R118W mutant from the patient allowed a comprehensive

study of the role of human TRAF3 in various PRR and B cell signaling pathways. R118W fibroblasts failed to produce the cytokines IFN-β, IFN-λ or IL-6 in response to TLR3 stimulation by either double-stranded RNA or the neurotrophic vesicular stomatitis virus, and in contrast to murine signaling pathways, NF-κB was strongly impaired, while IRF3 activation was only mildy affected (Figure 1). Furthermore R118W fibroblasts failed to control VSV replication as effectively as control cells. These defects were all complemented by transfection of wild-type TRAF3 into cells. The defects in PRR signaling were not confined to the TLR3 pathway, because gene induction by TLR7 in monocytes or by RIG-I in fibroblasts (Figure 1) was also inhibited in mutant cells. The induction of IFN-β and IFN-λ in response to viral infection of R118W fibroblasts was similarly suppressed. Surprisingly then, IFN-α induction in response to a range of viruses including HSV-1 was normal in R118W peripheral blood mononuclear cells (PBMCs), suggesting that residual levels of TRAF3 are sufficient for IFN responses in PBMCs, or that other proteins may compensate for the reduced TRAF3 in these cells. Thus similar to the murine data, human TRAF3 is important for IFN induction, although the particular transcription factors regulated by TRAF3 differ in humans, as might the cell-specific role of TRAF3 in virus-induced IFN.

Turning to R118W B cells, TNFR pathways (CD40 and BAFF) were affected both positively and negatively by TRAF3 loss of function. For CD40 TRAF3 is a positive regulator, resulting in the impaired production of some cytokines in R118W cells, whereas for the B cell activation factor receptor (BAFF-R) pathway, TRAF3 is a negative regulator (Figure 1). Similar to the situation in murine B cells, the noncanonical NF-κB pathway involving p100 processing into p52 was constitutively active due to impaired TRAF3 function (Figure 1). However, it is likely

that the overall effects of impaired TRAF3 function on B cell signaling are more minor than that on the PRR pathways because complete defects in CD40 or BAFF-R signaling do have clinical consequences in terms of B cell expansion and function, whereas no such consequences seemed apparent for the TRAF3-deficient patient studied here.

In terms of human TRAF3 function, several questions remain. Although the authors argue that the role of TRAF3 in protection against HSE is due to TRAF3's involvement in the TLR3 pathway (Figure 1), it is possible that there are alternative explanations for this. Certainly the TRAF3 deficiency mimics the previously reported TLR3 deficiency in terms of susceptibility to HSE (Zhang et al. 2007), and indeed TLR3 is a PRR particularly expressed in the CNS. However, HSV-1 has been shown to be sensed by multiple PRRs in mice, and thus an alternative explanation for the essential role of TRAF3 in HSE protection in humans could be due to TLR3-independent roles for TRAF3 in PRR signaling (for example Figure 1(c)). Then TLR3 and TRAF3 would both be independently necessary, but not sufficient, for protection against HSE. A further but related issue is, as in the case for TLR3, the narrow disease susceptibility arising from TRAF3 deficiency, especially given the prediction from murine and in vitro studies that TRAF3 is utilised by most PRRs for IFN induction. One explanation for this is that in humans the function of TRAF3 is redundant except for TLR3 signaling in the CNS, and that TLR3 is redundant in viral sensing except for neurotrophic viruses such as HSV-1. The exact mechanism by which TRAF3 (and TLR3) exert a protective effect against HSV-1 infection in the CNS is also unknown and requires further study. Further work will also be required to confirm that human TRAF3 is more important for NF-κB activation than for IRF3 activation, as this would be a crucial mouse-human difference. Overall, this study

demonstrates the importance of TRAF3 for human host defense and provides mechanistic insights into how this important protein regulates human immune signaling.

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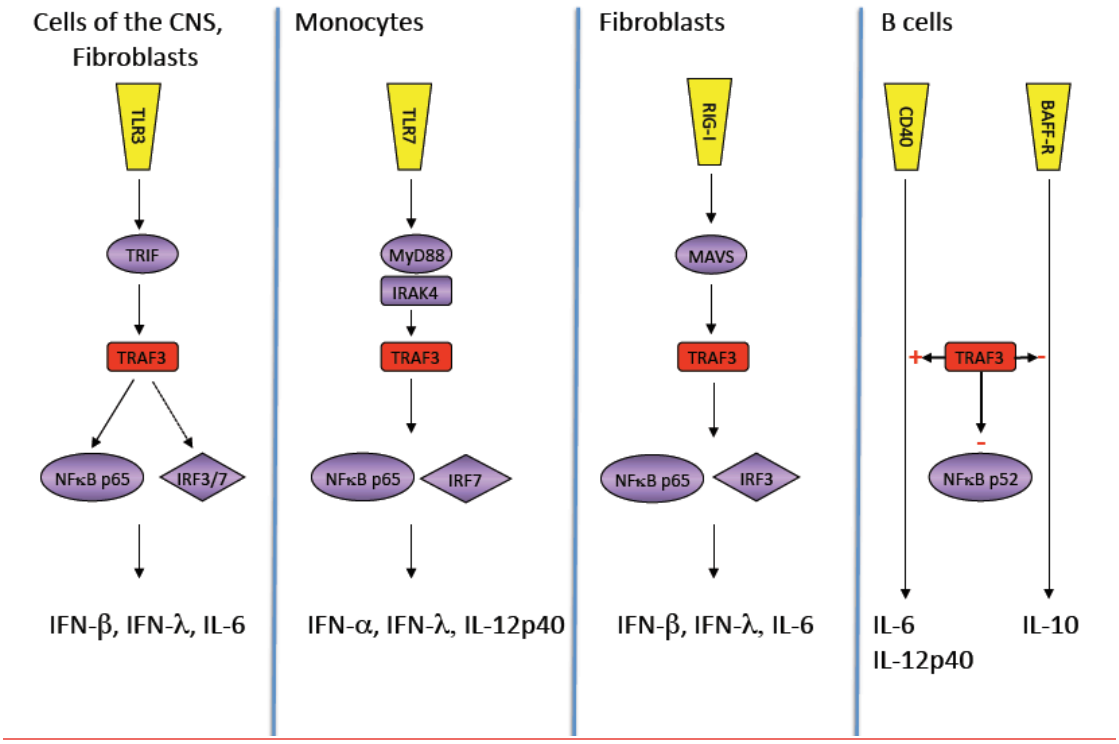

Figure 1 . Role for TRAF3 in human immune signaling pathways. Using *ex vivo* cells from a patient with an autosomal dominant TRAF3 deficiency, the contribution of TRAF3 to some human immune signaling pathways has been revealed. Functional TRAF3 was required for normal TLR3-, TLR7- and RIG-I-mediated gene induction, whereas in B cells, TRAF3 has both positive (+) and negative (-) roles in gene induction by TNFRs (CD40 and BAFF-R). As was shown for murine B cells, TRAF3 prevents constitutive processing of NFκB p100 into NFκB p52 in human cells. However in contrast to murine studies, which show a role for TRAF3 in IRF3 and not NFκB p65 activation, in human TLR3 signaling, TRAF3 has a major role (solid arrow) in p65 activation but more minor role (dashed arrow) in IRF3 activation (left panel). Genetic evidence suggests that the pathway depicted on the left primarily accounts for the requirement for TRAF3 in immunity against primary infection by HSV-1 in the CNS.