A Different TIPE of Immune Homeostasis

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Proteins with death effector domains (DED) are key signal transducers involved in cell death and inflammation. In this issue of *Cell*, Sun et al. (2008) describe TIPE2, a DED protein that negatively regulates both T cell receptor and Toll-like receptor signaling. These findings reveal a new element critical to the maintenance of homeostasis in both the adaptive and innate immune systems.

Homeostasis within the immune system is maintained by myriad mechanisms that include the regulation of immune cell activation and programmed cell death (Bidere et al., 2006). Altered regulation of cell death or activation and expansion of immune cells disrupts homeostasis of the immune system, which may result in potentially lethal inflammatory diseases. A subfamily of proteins that contribute to immune homeostasis possesses a hexahelical bundle motif, called the death effector domain (DED). The DED is structurally related to the death domain (DD) and the caspase activation and recruitment domain (CARD), which are found in proteins participating in cell death and other signaling pathways (Tibbetts et al., 2003). Homotypic interactions between DED proteins have been shown to regulate programmed cell death triggered by activation of Fas and other death receptors. For example, FADD links Fas to the cysteine proteases caspase-8 and caspase-10 through homotypic interaction between their DED domains to initiate apoptosis. By contrast, cellular and viral antiapoptotic DED proteins, known as FLIPs, inhibit Fas-induced death by interfering with FADD and caspase-8 (Table 1). However, it is now well accepted that several DED proteins also critically regulate cell proliferation in addition to cell death (Tibbetts et al., 2003) (Table 1). In this issue, Sun et al. (2008) add to the growing list of DED-containing proteins with their identification of a new member called TIPE2 (tumor necrosis factor-α-induced protein 8-like 2). These investigators show that TIPE2 governs immune homeostasis in both the innate and adaptive immune systems by negatively regulating signaling by T cell receptors and Toll-like receptors (TLRs).

TIPE2 was initially identified as a gene abnormally expressed in the inflamed spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. TIPE2 shares 53% amino acid identity with tumor necrosis factor (TNF)-α-induced protein 8 (TNFAIP8), a DED protein that may regulate apoptosis. The authors showed that at 2 months of age, TIPE2-deficient mice developed progressive immune pathology characterized by weight loss, splenomegaly, leukocytosis, and multiorgan inflammation leading to death. When challenged with bacterial (*Listeria monocytogenes*) or viral (lymphocytic choriomeningitis virus, LCMV) pathogens

Table 1. DED Proteins and Their Functions in Cell Death and Immune Cell Activation

Protein	Binding to Other DED Proteins	Role in Cell Death	Nonapoptotic Role
TIPE2	Caspase-8	Positively regulates Fas- and TCR-mediated death; not part of the DISC	Negatively regulates macrophage TLR2, TLR3, TLR4, and TLR9 signaling; $TIPE2^{-/-}$ mice are hypersensitive to LPS-induced septic shock
FADD	Caspase-8, MC159, PEA-15	Connects the death receptors to caspase-8 and caspase-10 to form DISC and initiate apoptosis	Thymocyte development, T cell proliferation, and cell-cycle control; positively regulates TLR3 signaling and modulates TLR4 signaling
Caspase-8	FADD, PEA-15, FLIP, TIPE2	Recruited to the DISC through FADD and propagates apoptosis	Necessary for T cell, B cell, and NK cell proliferation and TLR2, TLR3, and TLR4 signaling in B cells; cooperates with FADD to promote cell-cycle progression in T cells
$c\text{-FLIP}_L$	FADD, Caspase-8	Competes with caspase-8 for binding to FADD within the DISC and negatively regulates Fas killing	Involved in lymphocyte proliferation; cleaved by caspase-8 into an NF-κB activating fragment
$c\text{-FLIP}_S$	FADD, Caspase-8	Inhibits Fas killing	Reduces lymphocyte proliferation, caspase-8 activation, and NF-κB in T cells from transgenic animals
PEA-15	FADD, Caspase-8	Inhibits Fas killing	Sequesters ERK1/2 in the cytosol and promotes ERK-dependent phosphorylation of RSK
K13		Blocks Fas in vitro but not in vivo; protects TF-1 leukemic cells from cytokine withdrawal-induced death	Selectively activates NF-κB through binding to IKK; splenocytes from K13 transgenic mice exhibit enhanced proliferation following TCR and TLR4 stimulation; K13 transgenic mice present an increased risk of lymphoma

prior to the development of spontaneous disease, TIPE2-deficient mice showed increased numbers of $CD8^+$ T cells in the spleen and increased cytokine production, suggesting that TIPE2 regulates the T cell response. Consistent with this notion, TIPE2 was predominantly expressed in immune cells of the myeloid and lymphoid lineages. Notably, *TIPE2* expression was also induced by the cytokine tumor necrosis factor alpha (TNF-α) in at least one fibroblast cell line, suggesting that *TIPE2* may be expressed in many cell types to establish equilibrium during an inflammatory response. Intriguingly, there also exist two highly homologous and, as yet, uncharacterized proteins, TIPE1 and TIPE3.

Sun and colleagues found that TIPE2 played no role in B cell adaptive immunity but did modulate T cell receptor (TCR)-mediated activation of T cells. Although T cells lacking *TIPE2* were hyperactivated by TCR stimulation, there was no difference in their ability to proliferate compared to wild-type T cells. Paradoxically, *TIPE2* overexpression slightly repressed both lymphocyte activation and proliferation. These finding are intriguing given that lymphocyte proliferation is dramatically reduced in the absence of DED factors such as FADD, caspase-8, and $cFLIP_L$ (Chun et al., 2002; Zhang et al., 1998). In contrast, transgenic expression of the nonapoptotic DED factors $FLIP_L$ and FLIP K13 of human herpesvirus-8 promotes enhanced proliferation of lymphocytes (Chugh et al., 2005; Lens et al., 2002).

In the context of innate immunity, the authors found that TIPE2 negatively regulated the TLR signaling pathway. Macrophages lacking *TIPE2* or B cells stimulated with several TLR ligands produced more of the cytokines interleukin-6 (IL-6) or TNF-α and IL-1β, respectively, than wild-type cells. A dramatic difference in survival was also observed between mice with and without *TIPE2* that were treated with low-dose lipopolysaccharide (LPS) to induce septic shock. A common feature of these TLR signaling pathways is the formation of large multiprotein complexes necessary for signal transduction, and it is possible that TIPE2 may integrate into these complexes. The intracellular localization of TIPE2 in unstimulated and stimulated cells, as yet unexplored, could shed light on whether this is the mode of TIPE2 action.

How might these wide-ranging roles attributed to TIPE2 in modulating the immune response be mechanistically orchestrated? The authors first found that TIPE2 downregulated multiple signaling pathways in macrophages stimulated with LPS. Although TIPE2 did not target the extracellular signal-regulated kinase (ERK) pathway, it did repress activation of c-Jun N-terminal kinase (JNK) and p38 MAP kinase, resulting in diminished activity of the transcription factor AP-1. In addition, TIPE2 depletion led to increased nuclear translocation of the master transcription factor NF-κB subsequent to enhanced phosphorylation and degradation of its inhibitor IκBα. Sun et al. further found that a portion of TIPE2 was constitutively associated with proapoptotic caspase-8 and proposed that TIPE2's regulation of the NF-κB pathway may be mediated by this proapoptotic enzyme. However, it will be important to clarify the binding between TIPE2 and caspase-8 and to assess whether this interaction is an essential step in TIPE2 action.

Following on from their observation of TIPE2's interaction with caspase-8, Sun et al. also discovered another facet of TIPE2 function in regulating cell death. TIPE2 knockdown inhibited Fas-mediated apoptosis and antigen receptor-induced cell death (AICD), which partially involves Fas. In addition, ectopic expression of TIPE2 enhanced Fas-mediated killing. Surprisingly, although TIPE2 binds to caspase-8, TIPE2 was not found in the death-inducing signaling complex (DISC) following Fas ligation and did not impair FADD and caspase-8 recruitment. This distinguishes TIPE2 from previously characterized DED proteins that bind to FADD or caspase-8 and that alter the DISC. The mechanism by which TIPE2 inhibits apoptosis remains an open question.

DED proteins interact with many proteins that do not contain DED domains. For example, the DED protein PEA-15 associates with the ERK1/2 kinases to prevent their accumulation in the nucleus (Formstecher et al., 2001) and the K13 DED protein binds to the IKK complex to promote the activation of NF-κB (Liu et al., 2002). Furthermore, odd bedfellows such as FADD and CK1α, or caspase-8 and TRAF6, seem to pair up in functionally important ways. Therefore, it is likely that TIPE2 has binding partners that are not DED proteins. Defining these TIPE2-interacting partners will undoubtedly provide insights into how TIPE2 regulates immune signaling pathways. Although much work is required to fully elucidate how TIPE2 operates, the identification of this protein and the intriguing phenotype of the TIPE2-deficient mice establish TIPE2 as an important factor in the maintenance of immune homeostasis.

REFERENCES

Bidere, N., Su, H.C., and Lenardo, M.J. (2006). Annu. Rev. Immunol. *24*, 321–352.

Chugh, P., Matta, H., Schamus, S., Zachariah, S., Kumar, A., Richardson, J.A., Smith, A.L., and Chaudhary, P.M. (2005). Proc. Natl. Acad. Sci. USA *102*, 12885–12890.

Chun, H.J., Zheng, L., Ahmad, M., Wang, J., Speirs, C.K., Siegel, R.M., Dale, J.K., Puck, J., Davis, J., Hall, C.G., et al. (2002). Nature *419*, 395–399.

Formstecher, E., Ramos, J.W., Fauquet, M., Calderwood, D.A., Hsieh, J.C., Canton, B., Nguyen, X.T., Barnier, J.V., Camonis, J., Ginsberg, M.H., and Chneiweiss, H. (2001). Dev. Cell *1*, 239–250.

Lens, S.M., Kataoka, T., Fortner, K.A., Tinel, A., Ferrero, I., MacDonald, R.H., Hahne, M., Beermann, F., Attinger, A., Orbea, H.A., et al. (2002). Mol. Cell. Biol. *22*, 5419–5433.

Liu, L., Eby, M.T., Rathore, N., Sinha, S.K., Kumar, A., and Chaudhary, P.M. (2002). J. Biol. Chem. *277*, 13745–13751.

Sun, H., Gong, S., Carmody, R., Hilliard, A., Li, L., Sun, J., Kong, L., Xu, L., Hilliard, B., Hu, S., et al. (2008). Cell, this issue.

Tibbetts, M.D., Zheng, L., and Lenardo, M.J. (2003). Nat. Immunol. *4*, 404–409.

Zhang, J., Cado, D., Chen, A., Kabra, N.H., and Winoto, A. (1998). Nature *392*, 296–300.