cytes leading to lymphoproliferative disease. Therefore, lymphocyte homeostasis in vivo requires a tight balance between ongoing lymphocyte generation and lymphocyte death. The study by Lin et al. illustrates that lymphoproliferative disease can result from unrestrained lymphocyte activation and proliferation, possibly without any alterations in apoptosis. However, NF-κB exerts antiapoptotic effects secondary to new gene transcription, in addition to its important role in lymphocyte activation and proliferation. Thus, whether the apparent lack of apoptotic defects in Foxo3a-deficient mice results from a failure to induce NF-κB-dependent anti-apoptotic molecules, coupled with a failure to induce specific Foxo3a-dependent proapoptotic genes, remains unresolved. Delineating how different transcription factors form regulatory complexes for turning on programs of activation versus programmed cell death will shed further light on the regulation of lymphocyte homeostasis in vivo.

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SHIP, TGF-β, and Endotoxin Tolerance

Research into the biology of endotoxin (aka lipopolysaccharide; LPS) began well over 100 years ago (Pfeiffer, 1892) and has had many unexpected turns. The phenomenon of endotoxin tolerance was among these and has engendered immense curiosity over the years. Sly and colleagues (this issue of *Immunity*) have taken an important step toward understanding the phenomenon of endotoxin tolerance and have enhanced our comprehension of LPS signaling as a whole by demonstrating that the cytosolic phosphatase SHIP is required to permit the development of an LPS-refractory state in macrophages and mice.

Endotoxin tolerance was a phenomenon first noticed in humans rather than in animals. In the 1930s and 1940s, typhoid vaccine was administered to patients with neurosyphilis to induce fever, which was known to slow the progress of *T. pallidum* infection within the central nervous system. With repeated administration, the vaccine showed progressive loss of efficacy as a pyrogen, and an escalation of dose was required to achieve a therapeutic effect: a phenomenon closely investigated by Beeson (Beeson, 1947a). He established a rabbit model of tolerance and concluded that tolerance arose from an interaction between LPS and reticuloendothelial cells, since "reticuloendothelial blockade" with Thorotrast (colloidal thorium dioxide) or trypan blue could both eliminate tolerance and impair the clearance of the pyrogen from the blood (Beeson, 1947b). Noting the lack of serospecificity (tolerance prevented a response to pyrogens prepared from almost any gram-negative organism) and the recovery of competence to mount a febrile response within a few weeks after pyrogen injections were terminated (a time point at which antibodies against capsular polysaccharides were still abundant in serum), Beeson and others discounted a role for antibodies against the pyrogen in the development of endotoxin tolerance, considering instead that a cellular mechanism was at play. Later workers concluded that "endogenous pyrogens" (i.e., pyrogenic cytokines) were produced largely by mononuclear phagocytic cells in response to LPS; hence, it made sense to think that the ability to develop tolerance was an inherent property of these cells. The fact that LPS tolerance could be observed in cultured macrophages was a commonplace observation that reinforced this view. The discoveries that TNF (Beutler et al., 1985) and interferon-β (Karaghiosoff et al., 2003) mediate much of the toxicity of LPS have, in turn, given relevant endpoints to follow in assessing LPS tolerance both in vivo and in vitro.

The cellular mechanism for endotoxin tolerance is of obvious interest, because dampening the LPS response may have important clinical utility, most notably in severe gram-negative infections. Hence, the biochemical basis of the "natural" inhibition of LPS signaling that occurs in mononuclear phagocytes has attracted much attention. But for many years, the biochemical basis of LPS tolerance remained obscure and only now is a detailed understanding of the phenomenon beginning to emerge.

The identification of the LPS receptor, revealed as TLR4 by the positional cloning of the *Lps* locus (Poltorak et al., 1998), has offered a chance to probe various aspects of the LPS signaling pathway in an effort to decipher the mechanism of feedback inhibition. It has become clear that there are two major signaling pathways that emanate from the LPS receptor. One of these is the MyD88-dependent signaling pathway, which depends on the adapters MyD88 and Tirap. The other is the MyD88-independent signaling pathway, which depends on the adapters TRIF and TRAM. Both pathways lead to the activation of NF-κB via activation of TRAF-6. But the MyD88-independent pathway is unique in that it also leads to the activation of IRF-3 and to the production of type I interferons (Hoebe et al., 2003; Yamamoto et al., 2003).

Feedback inhibition might potentially occur at several points in these pathways. In mice, LPS stimulation causes a fall in TLR4-encoding mRNA in macrophages (Poltorak et al., 1998; Nomura et al., 2000), but this may be a species-limited phenomenon rather than a general one. IRAK-M, activated by traffic through the LPS signaling pathway, is known to have an inhibitory effect on MyD88-dependent LPS signaling (Kobayashi et al., 2002). SOCS-1 interferes with type I interferon signaling (Kinjyo et al., 2002). ST2, a TIR-motif receptor with immunoglobulin superfamily ectodomain repeats, blocks LPS signaling as well (Brint et al., 2004), probably through an interaction with other TIR motif proteins (TLR4 itself or one of the cytoplasmic TIR adaptor proteins). SIGIRR, another member of the same family of TIR motif receptors, has a similar effect (Wald et al., 2003). Ultimately, tolerance has been said to depend upon the formation of inhibitory p50 NF-κB homodimers, which block the transcription of inflammatory cytokine genes rather than enhancing it as p50:p65 heterodimers do (Ziegler-Heitbrock, 1995). Meanwhile, inhibitory cytokines such as IL-10, which are believed to downmodulate the response to LPS, are still expressed. Hence, LPS tolerance may be multifactorial. Nevertheless, a unitary "tolerance pathway" may exist; i.e., a single molecular event may initiate tolerance. If so, it would certainly be important to find it.

SHIP converts the PI3 kinase second messenger PI-3,4,5-P_3 to PI-3,4-P_2. It is strongly induced at the protein level by LPS and phosphorylated in response to LPS as well. In $SHIP^{+/+}$ cells, LPS tolerance is associated with inhibition of both NF-κB activation and STAT-1 activation: two of the principal endpoints of LPS signal transduction. In $SHIP^{-/-}$ cells, such inhibition does not occur, and tolerance is not observed. SHIP activation occurs later than IRAK-M and SOCS-1 induction after an LPS challenge. Nonetheless, as the authors point out, tolerance cannot occur in the absence of SHIP, suggesting a dominant requirement for the enzyme in mediation of the phenomenon (Sly et al., 2004 [this issue of *Immunity*]). The authors also suggest a vital role for TGF-β in the development of tolerance.

The topic of LPS tolerance is a very large one and cannot be fully elucidated in a single publication, though Sly and colleagues have made a significant advance. Among the questions raised by this important piece of work, we may wonder exactly how TGF-β is induced by LPS (i.e., via the MyD88/Tirap pathway or via the TRIF/TRAM pathway or both). Is crosstolerance (the development of tolerance to LPS induced by TLR2 ligands, for example) (Sato et al., 2000) dependent upon SHIP just as tolerance is? If so, we might conclude that only the MyD88-dependent pathway is important for SHIP activation. And why does SHIP-induced tolerance seem to supersede tolerance induced by IRAK-M and SOCS-1? Clearly there is room for much additional work now that a foothold is has been made.

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