EBV-infected B cells and decreased humoral response, may derive from the inability of SAP-deficient immune cells to form stable interactions. Because NKT cell development requires thymocyte-thymocyte interactions, it would be interesting to confirm that SAP deficiency in NKT cell development destabilizes thymocyte-thymocyte contacts.

Imaging studies of the immunological synapse (IS) by Zhao et al. (2012) and Kageyama et al. (2012) can potentially provide a mechanism for how SAP deficiency may lead to decreased conjugate stability. Both studies showed that Ly108 was recruited to the center of the IS of T cells conjugated to B cells. In the presence of SAP, SHP-1 was excluded from this central region. In contrast, in *Sh2d1a*$^{-/-}$ T cells, SHP-1 was present throughout the entire synapse. This affected the localization of proximal signaling events, such as Lck phosphorylation at the IS (Zhao et al., 2012). It is well known that localized signaling at the IS is critical for optimal downstream events such as cytoskeletal reorganization, polarization, and adhesion to the target cell. Therefore, local inhibition of signaling pathways by SHP-1 recruitment to the IS, as well as the absence of local positive signaling through SAP, can explain why SAP-deficient T cells can not form stable conjugates. Indeed, in the case of CD8^{+} T cells, SAP deficiency was associated with defective cytoskeleton reorganization (decreased clearance of the actin from the central region), leading to impaired T cell polarity (Zhao et al., 2012).

In summary, three papers in the current issue of *Immunity* show that SAP has a unique signaling mechanism that modulates both positive and negative signaling pathways through SLAM receptors (Figure 1). In the presence of SAP, this allows for the engagement of SLAM family members to have a steep amplifying effect on T, NKT, and NK cell activation, linking cell-cell adhesion with the signaling machinery. This forward feedback loop induced by SLAM family receptor adhesion could then enhance inside-out signaling by integrins, further enhancing conjugate formation, allowing for additional SLAM receptor engagement. Impaired contacts between cells can explain most of the immune defects observed in SAP-deficient mice and XLP1 patients. Further studies will be required to confirm that SAP deficiency also impairs lymphocyte interactions in XLP1 patients and to assess the relative contribution of SAP-regulated activating and inhibitory pathways through SLAM receptors in human immune cells.

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Bystanders Not So Innocent after All

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Interleukin 27 (IL-27) regulates immune responses, including T helper 17 (Th17) cell activity. In this issue of *Immunity*, Hirahara et al. (2012) demonstrate that IL-27 suppresses Th17 cells in *trans* through induction of the inhibitory ligand PD-L1 on bystander T cells.

The mammalian immune system utilizes an arsenal of diverse and powerful effector mechanisms to deal with a wide variety of pathogens. Without proper control, these mechanisms can not only kill bacteria and viruses, but also inflict serious damage, including death, to the host. It is therefore of vital importance that any given immune response is carefully balanced in a way that ensures both pathogen clearance as well as survival of the host. Negative regulation of the immune system is a field of intense research, not least because it holds great therapeutic potential: various forms of

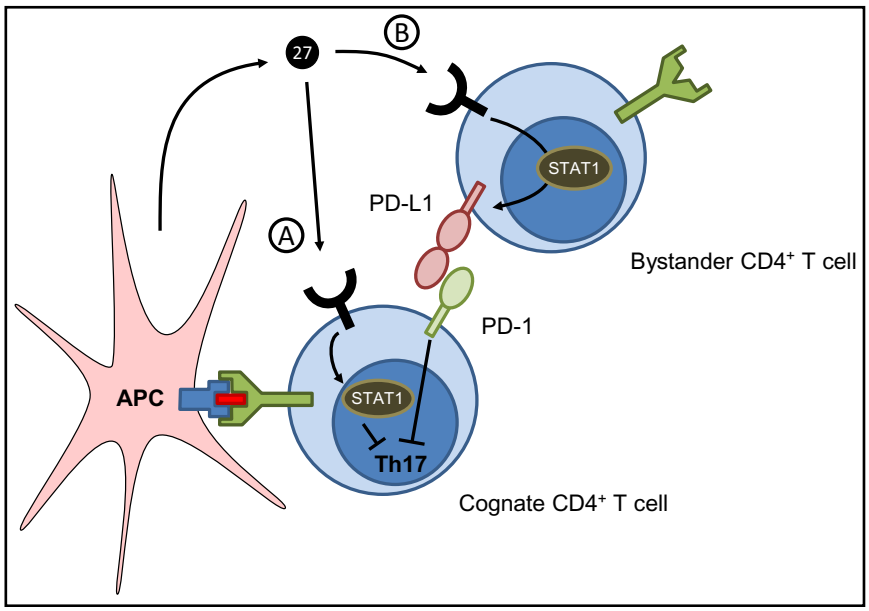

Figure 1. Schematic Representation IL-27-Dependent Th17 Cell Suppression
Cognate T cells recognize antigen presented by APC and will clonally expand. Bystander T cells do not recognize antigen and will not expand but will still outnumber cognate T cells.
(A) Direct suppression elicited by binding of IL-27 to its receptor on the developing Th17 cell is a STAT1-dependent effect.
(B) Indirect effect discovered by Hirahara et al. (2012) involves STAT1-dependent upregulation of PD-L1 on bystander T cells, which then signal for Th17 cell suppression through the PD-1 receptor expressed on the cognate T cell.
APC, antigen-presenting cell; 27, interleukin-27; Th17, T helper cell type 17; STAT1, signal transducer and activator of transcription 1; PD-1, programmed death-1; PD-L1, PD-1 ligand 1.

autoimmune, inflammatory, and allergic disease represent failures of negative immune regulation; conversely, immune responses appear to be inappropriately suppressed in the context of chronic viral infection and cancer.

Interleukin-27 (IL-27) is an antigen-presenting cell (APC)-derived heterodimeric cytokine consisting of p28 and EBI3 subunits, which signals through a receptor expressed on virtually all immune cells. Although IL-27 can promote inflammation in some in vivo models, its predominant in vivo role appears to be in restraining Th1, Th2, and Th17 cell-driven immune responses. This immunoregulatory function of IL-27 is illustrated by mice deficient in the IL-27 receptor alpha chain (IL-27Ra), which suffer from immune pathology but not compromised pathogen clearance in the context of a number of Th1 and Th2 cell-dependent infectious agents (Batten and Ghilardi, 2007; Stumhofer and Hunter, 2008). Furthermore, *Il27ra*$^{-/-}$ mice display exacerbated susceptibility to disease in the Th17 cell-driven experimental autoimmune encephalomyelitis (EAE) model (Batten et al., 2006; Stumhofer et al., 2006).

Most of the anti-inflammatory functions of *Il27ra*$^{-/-}$ mice rely on signal transducer and activator of transcription-1 (STAT-1) activation but are independent of its capacity to elaborate production of interferon-γ (IFN-γ). Yet the precise mechanism by which IL-27 constrains immune responses in vivo has remained elusive. In this issue of *Immunity*, Hirahara et al. (2012) illuminate a previously unappreciated mechanism of action of IL-27, showing that it can suppress Th17 cell differentiation in *trans* through STAT-1-mediated induction of PD-L1 on bystander, noncognate T cells (Figure 1).

Hirahara et al. (2012) used an experimental system in which naive T cells were stimulated with recombinant IL-27 (without TCR ligation) and then washed. Their effect on the activation and cytokine production of admixed T cells was then assessed. In such a system, IL-27-primed T cells inhibited Th17 cell but not Th1 cell differentiation in *trans*. Remarkably, IL-27-primed T cells conferred substantial protection against EAE when cotransferred together with 2D2 transgenic T cells into naive recipients prior to myelin oligodendrocyte glycoprotein (MOG) immunization. Again, this effect involved suppression of IL-17 (but not IFN-γ) production by the MOG-specific 2D2 T cells. Antigen specificity or even antigen priming of the IL-27-primed T cells was not required, suggesting that nonspecific bystander T cells can limit Th17 cell differentiation of antigen-specific effector cells in *trans*.

To determine the mechanistic underpinnings of this effect of IL-27, the authors profiled IL-27-induced gene expression in T cells by microarray. This experiment led to the identification of a number of IL-27-induced genes and among the highest ranking was programmed death ligand 1 (PD-L1, also known as B7-H1 or CD274). PD-L1 and PD-L2 (B7-DC; CD273) are members of the B7 family of costimulatory ligands that bind to the inhibitory receptor programmed death-1 (PD-1), which is expressed on T cells after activation. It is well understood that signals received by the T cell through PD-1 suppress activation after T cell receptor (TCR) signaling through inhibition of PI3K activity, blocking T cell proliferation and inhibiting cytokine production. Expression of PD-L1 and PD-L2 in tissues is crucial for peripheral tolerance, as is impressively illustrated by mice deficient in PD-1, which suffer from spontaneous autoimmunity in susceptible genetic strains and from exacerbated pathology in T cell-mediated autoimmune disease models (Fife and Pauken, 2011). Subsequent validation experiments by Hirahara et al. (2012) included antibody-mediated blockade or genetic deletion of PD-L1 signaling, both of which neutralized the ability of IL-27-primed cells to execute Th17 cell suppression in *trans*. Furthermore, recombinant PD-L1 mimicked the effect of IL-27-primed T cells. Together, these results suggest that PD-L1 is both necessary and sufficient for Th17 cell suppression by bystander T cells in *trans*.

Consistent with earlier observations that STAT-1 can induce PD-L1 expression in T cells (Loke and Allison, 2003), IL-27 was also found to act through STAT-1 in this context. Furthermore, other STAT-1-activating cytokines, such as interferons (IFN) α, β, and γ, also induced PD-L1 but did so more broadly than IL-27, the effect

of which was limited to T cells. This apparent specificity is puzzling, because it is well documented that B and myeloid cells are fully capable of responding to IL-27. It is therefore suggested that mechanisms exist to modulate the capacity of IL-27 to induce PD-L1 in a cell type-specific fashion.

An important implication of this study is that noncognate bystander $CD4^+$ T cells can drastically influence the differentiation of the antigen-specific response. This has potentially wide reaching implications for how we understand the control of T helper cell activation and differentiation. Presumably both cognate and noncognate T cells are subject to IL-27 signals under inflammatory conditions. The authors argue that because of the overwhelming ratio of naive:antigen-specific $CD4^+$ T cells, the in *trans* effects are likely to be important. However, proving the relevance of the IL-27-STAT-1-PD-L1 axis definitively in a physiological context is a formidable challenge. We must not forget that PD-L1 expression is potently induced by TCR ligation already (Yamazaki et al., 2002), and, as the authors and previous literature show, by STAT-1-inducing cytokines other than IL-27. Furthermore, IL-27 can directly suppress Th17 cell differentiation in the context of TCR ligation, and therefore this effect is probably PD-L1 independent (Batten et al., 2006). Although Hirahara et al. (2012) relied heavily on ex vivo-manipulated T cells to allow for careful dissection of the mechanism, they nevertheless took a first step toward addressing this problem by interrogating PD-L1 expression in mice deficient for the Ebi3 subunit of IL-27. Indeed, T cells in *Ebi3*$^{-/-}$ mice failed to express PD-L1 upon infection with *T. gondii.* Although genetic deletion experiments abrogate all effects of IL-27, including direct ones, these data suggest that the IL-27-PD-L1 axis may indeed be physiologically important. More sophisticated genetic models and the use of radiation chimeras will be required to unambiguously define the physiologic importance of IL-27-elicited and PD-L1-mediated in *trans* suppression in vivo.

Beyond IL-27, Hirahara et al. (2012)'s data may also prompt mechanistic studies on IL-35, a recently described cytokine that is closely related to IL-27 and shares the EBI3 subunit. A body of literature exists suggesting that stimulation of T cells with IL-35 turns them into suppressive iTr35 cells (Belkaid and Chen, 2010). The mechanism by which this happens has not been fully elucidated, and the reported in vivo data bear striking resemblance to Hirahara et al. (2012)'s results. Given IL-35's close relationship to IL-27, as well as its capacity to activate STAT-1, PD-L1 now has to be considered a prime suspect to mediate the effects of IL-35.

IL-27Ra deficiency results in exacerbated Th1, Th2, and Th17 cell responses in vivo; on the other hand, in *trans* suppression by the IL-27-STAT-1-PD-L1 axis was limited to Th17 cell responses and did not affect IFN-γ production at all. It thus appears that the current study cannot explain why Th1 cell responses are exacerbated in *Il27ra*$^{-/-}$ mice, and Th2 cell responses were not studied. However, the focus of the present study was on naive cells differentiating into an effector phenotype, which reflects only the beginning of an immune response. It is still possible that the immune pathology and exacerbated production of IFN-γ seen in *Il27ra*$^{-/-}$ mice infected with *T. gondii* is due to the abrogation of PD-L1 expression at a later stage during the disease progression, especially because the pathology does not manifest until several days postinfection (Stumhofer and Hunter, 2008). This hypothesis can now be tested.

Certainly, the IL-27-STAT-1-PD-L1 axis of immune suppression will not dominate under all circumstances. After all, IL-27 is known to be pathogenic rather than protective in murine models of lupus, proteoglycan-induced arthritis, and transfer colitis (Wojno and Hunter, 2012). These seemingly contradictory observations are easily reconciled by the notion that the net outcome of any given in vivo situation always reflects the sum of all activating and inhibitory immune mechanisms. With their elegant series of experiments, Hirahara et al. (2012) uncovered a mechanism by which IL-27 can suppress T cell responses through noncognate, bystander T cells. Undoubtedly, this work will prompt many follow-up studies to determine whether and to what extent this mechanism actually plays in the context of various immune challenges and diseases.

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