IL-2: Fine-tuning the Germinal Center Reaction

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T follicular cells help B cells to drive germinal center formation. In this issue of *Immunity*, Ballesteros-Tato et al. (2012) demonstrate that high amounts of interleukin-2 inhibit production of this critical T effector subset.

Antigen-specific long-lived plasma and memory B cells originate in germinal centers (GCs) and provide effective and lasting protection against infection. The GC reaction is controlled by antigen-specific cognate interactions between T follicular (Tfh) and B cells bordering the T cell zone and the follicle (Vinuesa and Cyster, 2011). The migration of T and B cells to these locations and subsequent intercellular interactions is facilitated by upregulation of the chemokine receptor CXCR5. Importantly, antigen-priming and costimulation of $CD4^+$ T cells, the latter through engagement of CD28, ICOS, and CD40L on the T cell with CD80 and CD86, ICOSL, and CD40 on DCs and B cells, contribute to the upregulation of the transcription factor Bcl-6 (Figure 1), a signature regulator promoting the development of Tfh cells (Crotty, 2011). The Tfh-helped B cells or centroblasts proliferate and express activation-induced cytidine deaminase to undergo somatic hypermutation (SHM) in the V region of their B cell receptor (BCR). This process produces B cell clones that bind the immunizing antigen with higher affinity and are selected to survive by interaction with the antigen-specific Tfh cells in the GCs. Thus, GC formation is a highly regulated process involving a large number of molecular interactions that promote differentiation of Tfh and B cells necessary for the production of high-affinity isotype class switched antibodies as well as immunological memory (Figure 1).

In the current issue of *Immunity*, Ballesteros-Tato et al. (2012) show that the amounts of interleukin-2 (IL-2) in vivo also importantly influence whether developing $CD4^+$ T effector cells choose a Tfh cell fate. The key point is that an environment low in IL-2 favors Tfh cells. The main approach used was to follow the immune response in mice infected with influenza virus that were untreated or received twice daily injections of IL-2 for 3 weeks. When these IL-2-treated mice were examined, they showed lower numbers of influenza-specific IgG secreting B cells. Their draining lymph nodes exhibited decreased GC structures that contained a nearly 80% reduction in B cells that expressed markers (PNA^{hi} Fas^+) characteristic of GCs and were specific for the influenza nucleoprotein (NP). To examine the cellular basis of this response, the authors generated chimeras by reconstituting lethally irradiated mice with equal numbers of congenic-marked bone marrow from WT or IL-2 receptor-deficient ($Cd25^{-/-}$) donors. When these reconstituted mice were infected with influenza, treatment with IL-2 had no effect on the B cell compartment, i.e., GC-derived B cells that produced NP-specific IgG antibodies were readily detected and these were equally distributed between WT and $Cd25^{-/-}$ B cells. However, when the T cell compartment was examined, a 3- to 4-fold increase was noted in the numbers of PD-1^+ $CXCR5^+$ NP-specific Tfh cells of $Cd25^{-/-}$ origin. Examination of NP-specific T cells from normal mice infected with influenza showed that Tfh cells expressed low amount of CD25, consistent with cells that received low IL-2R signaling and were characterized by high expression of Bcl-6 and ICOS. These latter two molecules on NP-specific T cells were both reduced in the presence of IL-2. Thus, increasing IL-2 did not intrinsically alter the B cell compartment but rather affected Tfh cells.

IL-2R signaling activates three main signaling pathways in T cells: MAPK, PI3K, and STAT5. Recent work has shown that IL-2-dependent activation of STAT5 is largely responsible for negatively regulating Tfh cell development (Johnston et al., 2012). This finding is highly reminiscent of the inhibitory role of IL-2 in the generation of T helper 17 (Th17) cells through STAT5 activation (Laurence et al., 2007). With respect to Tfh cells, activated lymphocytic choriomeningitis virus (LCMV)-specific T cell receptor transgenic $CD4^+$ T cells were engineered in vitro to overexpress active STAT5 or to lack STAT5 expression. When such cells were transferred to mice and infected with LCMV, increased STAT5 activation was associated with lower Tfh cells whereas lower STAT5 supported increased Tfh cell development and GCs (Johnston et al., 2012). Antigen-activated T cells expressing low amount of Blimp-1 (encoded by *Prdm1*), a repressor of Bcl-6, also supported Tfh cell development, further implicating regulation of Bcl-6 in this process.

The data from these two papers (Ballesteros-Tato et al., 2012; Johnston et al., 2012) support a model in which the amount of IL-2, STAT5, Blimp-1, and Bcl-6 are linked not only to the decision to adopt a Tfh cell fate but also to other T effector subsets and memory cells (Figure 1). During a specific immune response in which IL-2 expression is relatively high, IL-2-dependent STAT5 activation is more prolonged, supporting STAT5-dependent activation of Blimp-1. High amounts of Blimp-1 are associated with terminal differentiation of several cells types, including plasma cells and T effector cells. For T cells, high IL-2 and Blimp-1 are associated with production of short-lived CTL, Th2 cells, and a subpopulation of activated Treg cells. In settings where IL-2 is limited, Blimp-1 is not substantially expressed because of low activation of STAT5, thus

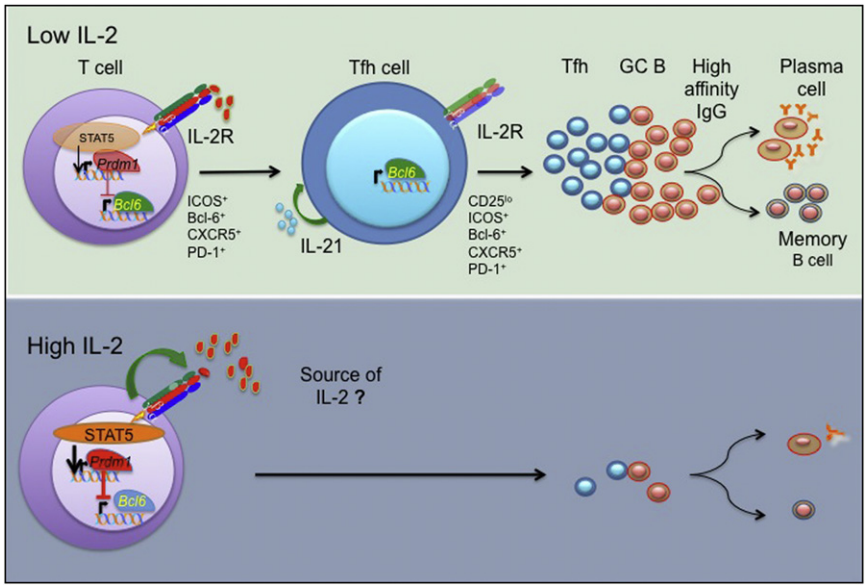

Figure 1. High IL-2 Expression Opposes Tfh Cell Development
Under low IL-2 conditions (top), antigen-activated CD4$^+$ T cells express high amounts of CXCR5, ICOS, Bcl-6, and PD-1 and efficiently develop into Tfh cells. CD25 expression in Tfh cells is relatively low, indicative of a cell encountering low IL-2. Tfh cells promote GC formation within the B cell follicles. These transient structures facilitate B cell differentiation, including production of high-affinity isotype switched IgG antigen-specific antibodies and memory B cells. In contrast (bottom), high IL-2 expression, via STAT5-dependent upregulation of Blimp-1 (*Prdm1*), represses the Tfh cell signature transcription factor Bcl-6 (*Bcl6*) to block the development of Tfh cells. Lower production of Tfh cells impairs the GC reaction and maturation of humoral immune responses. Thus, high IL-2 amounts interfere with the GC reaction and an effective antibody response.

promoting higher Bcl-6 expression that supports Tfh cell development and CD8$^+$ T cell memory commitment. However, amounts of Blimp-1 and Bcl-6 probably only partially explain these basic and critical cell fate decisions during an immune response. For example, IL-2-dependent STAT5 activation also opposes Th17 development. However, the available evidence suggests that Th17 cell fate is dictated by the transcription factor RORγt rather than expression of Bcl-6 and Blimp-1. In a related manner, although IL-2 and STAT5 positively support Treg cells and CTL, comparatively lower amounts of IL-2 support Treg cell development and homeostasis (Yu et al., 2009). Thus, the availability of IL-2 may represent a critical rheostat that is linked to major cell fate choices dictating the types of effector T cells while also supporting T memory cell development.

Expression of IL-2 is tightly controlled transcriptionally and posttranscriptionally. It was generally believed that this high regulation is required to critically control the magnitude of an immune response. However, both these studies (Ballesteros-Tato et al., 2012; Johnston et al., 2012) add to the growing body of evidence that substantial expansion of antigen-activated T cell clones can occur in the complete absence of IL-2. Further, these types of responses remained armed to control an infection because development of Tfh and Th17cells do not require IL-2 and other effector responses still somewhat develop independently of IL-2 in vivo. The exquisite control of IL-2 may actually represent a fundamental mechanism to fine-tune the type and magnitude of T effector and memory cells and consequently antigen-specific antibody responses.

The interworkings of an immune response are probably much more subtle than the nonphysiological approaches used to uncover IL-2-dependent STAT5 repression of Tfh cells in which IL-2 was systemically applied for a considerable time after viral challenge or where the STAT5 pathway was substantially altered in activated T cells (Ballesteros-Tato et al., 2012; Johnston et al., 2012). There remain several key missing links to better understanding IL-2 in this role of dictating cell fate choices. One fundamental issue is that we still have a very rudimentary understanding of when and where IL-2 is produced in vivo during an ongoing immune response to regulate Tfh and Th17 cell development versus supporting Treg cell homeostasis. Thus, it will be critical to identify the cell type(s) that produce IL-2 and amount of IL-2 in the natural microenvironment where T cells border the GCs and within the GCs where Tfh and B cells interact. Another key point is that the role of Treg cells in regulating the GC reaction is likely to be complex. Activated Treg cells reside in the GC and act to limit Tfh cell numbers (Chung et al., 2011; Linterman et al., 2011). However, Treg cells may also lower the available IL-2 by preferential consumption through their high expression of IL-2R to provide an environment to favor Tfh cell development. In this context, although the repression of Tfh cell development by IL-2 is T cell intrinsic, depletion of Treg cells exaggerated the capacity of exogenous IL-2 to oppose Tfh cell production probably by providing surplus IL-2 (Ballesteros-Tato et al., 2012). Future work must resolve whether the physiological role of Treg cells is to inhibit or promote Tfh cell production. Alternatively, both mechanisms may operate but in a contextual and/or a temporal distinct manner. Lastly, although lowering IL-2-dependent activation of STAT5 in T effector cells is critical for their development into Tfh cells, IL-2 also readily activates the MAPK and PI3K pathways in conventional activated T cells. The extent that these pathways might influence these cell fate choices requires further study.

These new findings have important implications for exquisite control of IL-2 to prevent pathological consequences during an immune response as well as for use of IL-2 in therapeutic settings. The efficacy of T cell-dependent humoral immunity is facilitated by Tfh cell help by promoting SHM, a process involving DNA recombination. Therefore, the maturation of Ab responses predisposes the host to the risks of autoimmunity by altering BCR binding that may recognize self-antigens and malignancies as a result of chromosomal translocations associated with DNA recombination events. Thus, precise amounts of local IL-2 may provide a mechanism to prevent these potential pathological consequences of

SHM in the GC by limiting the development and expansion of Tfh cells. In the context of IL-2 therapy, much emphasis in the past has been placed on boosting immunity, particularly in individuals with cancer or HIV-AIDS, but with limited success. Besides boosting Treg cells and immunosuppression, other unintended consequences of adjuvant IL-2 therapy that might compromise its efficacy are reduced Th17 cell and antibody responses, the latter through interfering with Tfh cell development (Figure 1). Thus, elevated IL-2 may be dually immunosuppressive by preventing development of Tfh and Th17 cells as well as by expanding Treg cells. Alternatively, low-dose IL-2 therapy was effective in preventing and reversing autoimmunity in preclinical mouse models and in ameliorating severe symptoms associated with graft versus host disease and hepatitis c virus-induced vasculitis in limited clinical testing (Koreth et al., 2011; Saadoun et al., 2011). Although these studies have focused on the positive effects of IL-2 on Treg cells, the benefit of this therapy may also reflect the blockade of Th17 and Tfh cells. These effects of IL-2 should now also be considered when IL-2 is used in the clinic.

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