Immunity
Commentary

The Roaring Twenties

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New cell types and cytokines have emerged as key participants in the elaboration of and recovery from inflammation. A collection of reviews covers recent advances in our understanding of this crucial component of host defense.

> "There is no confusion like the confusion of a simple mind."
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> —F. Scott Fitzgerald, *The Great Gatsby*, 1925

Cytokines have come a long way since the initial description of macrophage inhibitory factor in the 1960s (David, 1966; Bloom and Bennett, 1966). For years, immunologists marveled over the potency of these molecules to push, pull, and otherwise cajole a myriad of cells to do just about anything in various 96-well experimental settings. The therapeutic advances of anti-tumor necrosis factor (TNF) therapies in chronic inflammatory bowel and joint diseases and of interferon-α (IFN-α) in multiple sclerosis and hepatitis C virus infection moved the field beyond the broadband approaches epitomized by the use of steroids and broad-acting immunosuppressives, however, and demonstrated that more precise targeting of the immune system was possible. Here, understanding the language of the cytokines will be imperative. Various colony-stimulating factors have been exploited for bone marrow reconstitution (not without their own toxicities), but otherwise the staggering multiplicity of actions has cut short the broad use of these agents in human disease. Most of the cytokines exist within multiple family members and share combinatorial subunits and/or receptor-recognition modules, suggesting that evolution likes these things but also that nuanced differences probably exist among closely related family members and that these must be recognized before the potential therapeutic uses of these cytokines can be optimized. As updated by Scheinecker et al. among the series of reviews that follow, therapeutic targeting of interleukin-1 (IL-1), IL-6, and additional biomodulators extends the pharmacopoeia of immune intervention in inflammatory diseases, but precisely how to organize the rational approach for the use of anticytokine therapies remains too often empiric (Scheinecker et al. [2008], this issue of *Immunity*).

The series of review articles in this issue address recent subjects of much currency in the cytokine field. Of note, the discovery of additional $CD4^+$ T cell subsets that round out the canonical T helper 1 (Th1) and Th2 cell subsets first described in the 1980s emerges as a constant theme (Figure 1). Because of their capacity to become long-lived memory effector cells, these various populations of helper T cells can underpin many chronic inflammatory states mediated by cytokines when left unregulated. The Th1 and Th2 paradigm drove substantial discoveries in the immune system, perhaps most importantly the linking of epigenetic alterations at sites of expressed cytokines with canonical "master transcriptional regulators" whose binding could drive cell-fate decisions, thus providing a mechanism by which gene expression and cellular functions become stabilized (reviewed in Ansel et al., 2006; Lee et al., 2006). These findings in turn were driven by a quiet revolution in immunology. Over the past 20 years, 96-well-plate experiments were replaced by challenges using pathogens of every sort to tweak living animals in order to probe the functions of cells and, indirectly, the cytokines they secreted, in vivo. A slew of model organisms on defined genetic backgrounds became established, such that today's second-year immunology graduate students are quite conversant in experiments with LCMV, Listeria, helminthes, and protozoa. Close behind were autoimmune models of every organ. Once these models were in hand, investigators cooked up a myriad of lineage- and function-marking mice that could reveal cell fate and/or function in vivo, and, with that, the age of immunobiology expanded tremendously. Some of the fruits of those studies have been comprehensively summarized in the following reviews.

Two reviews in this issue, by Ouyang et al. (2008) and McGeachy and Cua (2008), outline in detail the discovery, function, and characterization of Th17 cells, including their potential role in the maintenance of barrier homeostasis in response to epithelial injury but also in the mediation of chronic inflammatory syndromes. Initially implicated by their dysregulated appearance in murine models of inflammatory diseases, including experimental autoimmune encephalomyelitis and antigen-induced arthritis, Th17 cells have gained favor with their capacity to restrict infections due to various extracellular bacteria and fungi. As covered in the reviews that follow, this has a certain credence, given that IL-17A and IL-17F, major cytokines produced by Th17 cells, induce granulocytopoiesis and CXC chemokines that together drive the accumulation of myeloid cells in tissues. The capacity of IL-22, a cytokine expressed by Th17 cells, to activate epithelial repair and defense responses suggests the working hypothesis that IL-22 plugs the holes in the barrier while IL-17 calls in the neutrophils to clean up the mess. In the mouse, problems seem to arise when Th17 cells get activated in closed spaces, such as in joints and in the brain, where neutrophils cannot be so readily disposed of. As addressed in both reviews, IL-17 and Th17 cells have been observed at the scene of the crime in diverse inflammatory syndromes in humans, including psoriasis, multiple sclerosis, asthma, and inflammatory

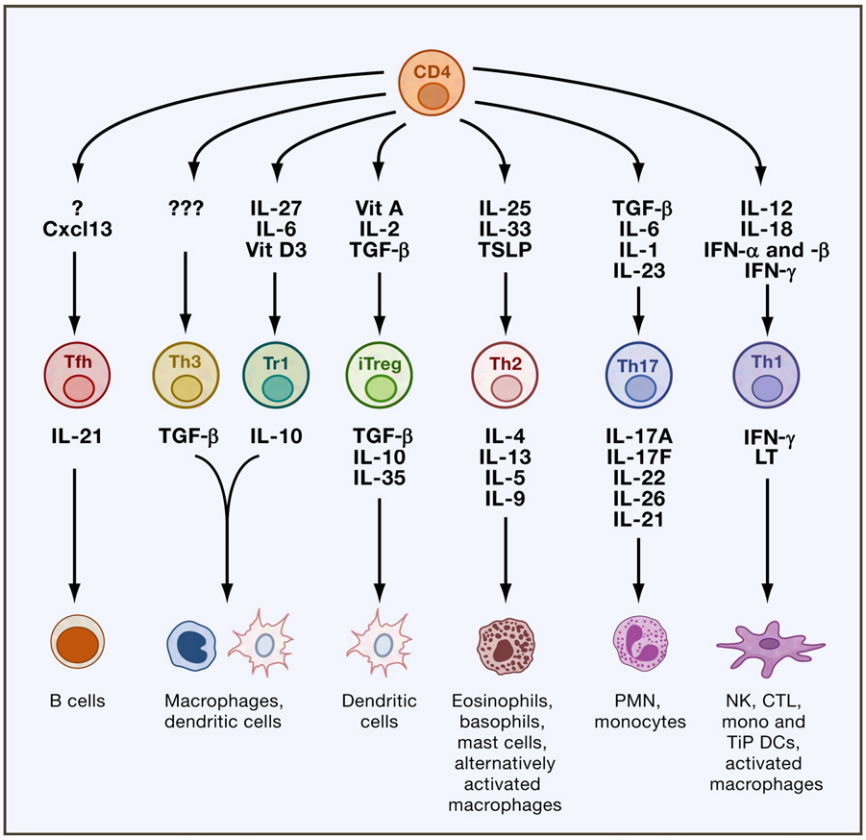

Figure 1. CD4⁺ T Helper Cell Subsets Orchestrate Inflammation and its Regulation

$CD4^+$ T cells in peripheral lymphoid tissues can adopt multiple cell fates that regulate inflammation. Some of the cytokines and other factors that have been associated with induction of each subset are indicated, although their roles in vivo remain incompletely defined. Each subset makes a canonical group of cytokines that act on other cells to drive interactions of each subset with other immune cells often found in close association. The capacity for memory allows T helper cell subsets to mediate chronic inflammatory states when not regulated.

Abbreviations are as follows: CTL, cytotoxic T cells; iTreg, inducible T regulatory cells; LT, lymphotoxin; PMN, polymorphonuclear neutrophils; Tfh, T follicular helper cells; TiPDCs, TNF- and iNOS-producing dendritic cells; TSLP, thymic stromal lymphopoietin.

bowel disease. Mutations in the receptor for IL-23, a cytokine that sustains the actions of Th17 cells in vivo, have been associated with altered risks for inflammatory bowel disease, psoriasis, and autoimmune thyroiditis. Details remain incomplete, including the particulars regarding whether the same factors are necessary in mouse and human for generation of Th17 cells and exactly which cell types contribute IL-17 under diverse conditions, but the associations of these cells with a multitude of inflammatory syndromes seems well established.

Inflammation demands regulation. As reviewed in this issue by Li and Flavell (2008), transforming growth factor-β (TGF-β) and IL-10 figure prominently in immunoregulation, as revealed years ago by the spontaneous inflammatory syndromes that erupt when either is deleted in the mouse. TGF-β is a requisite growth factor for $FoxP3^+$ T regulatory (Treg) cells, and the inflammation that arises in TGF-β null mice can be largely explained by the absence of these cells. TGF-β exists in a latent pro-form in tissues, where it can be activated by αvβ8 integrin on tissue dendritic cells (DCs) to sustain Treg, as best worked out in the lamina propria of the small bowel (Travis et al., 2007). So how do we activate the immune system if Treg cells are always in play? When Toll-like receptor ligands or inflammatory cytokines activate tissue macrophages, DCs, and epithelia, IL-6 is released, and the combination of TGF-β and IL-6 promotes the differentiation of Th17 cells. Intriguingly, the source of the TGF-β is T cells, probably Tregs themselves, suggesting an intimate dance between suppressor and inflammatory T cells that has been borne out by their close functional relationship during differentiation (Bettelli et al., 2006). Yet additional layers of regulation exist, and the addition of IL-27, which is made by DCs, with TGF-β and IL-6, leads to the elaboration of IL-10 from T cells, which, as reviewed by O'Shea and Murray, activates a potent anti-inflammatory program via Stat3 that includes induction of Socs3, a negative regulator of cytokine-receptor signaling. The complex layering of signals relayed by the module of Stat3 and Socs 3 hints at further regulatory nuances in the cell that exist to deconvolute cytokine-rich environments within the nucleus of individual cells but that ultimately determine how inflammatory responses are curtailed and homeostasis is recovered (O'Shea and Murray [2008], this issue).

As shown in the accompanying figure, alternative fates can await the helper T cell, although many remained incompletely defined as compared to the now-canonical Th1, Th2, Th17, and inducible Treg cells. The identification of "master regulators" for these cells—Tbet, GATA3, RORγt and RORα, and FoxP3, respectively—led to the use of reagents critical for the more complete understanding of their roles in immunity. T follicular helper (Tfh) cells mediate the germinal-center reaction, and understanding of the factors that drive their selection and differentiation could greatly aid the ability to make high-affinity antibody responses and long-lived memory B cells (King et al., 2008). Questions remain about the relationships of Tfh cells to canonical cytokine-expressing subsets, including the role of IL-21, which is a critical marker for Tfh cells yet also performs an autocrine function in the promotion of Th17 cell development. Despite their association with peripheral purulent inflammation, Th17 cells have been implicated in germinal-center reactions, an area that requires more study to be put into biologic perspective (Hsu et al., 2008). MicroRNAs (miR), as in other tissues, undoubtedly play a role in T helper cell subset development, and an unusual wrinkle has been shown with Tfh cells. In following up a mutation that revealed exuberant Tfh development and autoimmunity, Goodnow, Vinuesa and colleagues demonstrated that Roquin

plays a role in regulation of the capacity of miR-101 to target the inducible costimulator (ICOS) mRNA for degradation, thus limiting Tfh development and adding further complexity to the issue of regulation (Yu et al., 2007). Antibodies undoubtedly contribute to many chronic inflammatory syndromes, and understanding of the factors that regulate Tfh cell versus alternative T helper cell fate decisions will be an important area for further inquiry. Additional cell types, including IL-10-producing Tr1 cells and TGF-β-producing Th3 cells, await clarification via molecular elucidation of their lineage, localization, and stability in vivo.

Inescapable in this collection of informative and up-to-date reviews is the explosion of information regarding the IL-20s. Although not all are touched upon here, each has revealed an exceptionally rich biology that is suggestive of the Roaring '20s of American history, a time of unprecedented exploration, expansion, individualism, and creativity. Several, including IL-20, IL-22, IL-24, and IL-26, are members (with IL-10 and IL-19) of the IL-10 family of cytokines. Many of these cytokines have already been implicated in epithelial inflammatory syndromes in humans, such as psoriasis, although whether these serve to limit inflammation or promote it awaits further study, as conflicting information, as noted in the accompanying reviews, is apparent from the initial mouse experiments. The gene encoding IL-26, a prominent part of the human Th17 cell-derived cytokine repertoire, sits in a linked expression array with the genes encoding IL-22 and IFN-γ, although it has been disrupted by a long interspersed nuclear element insertion in the mouse. The mechanisms that regulate expression of this collection of genes have only begun to be probed (Schoenborn et al., 2007), but will likely bear similarities with the extensively mined Th2 cytokine locus comprising the genes encoding IL-4, IL-5, and IL-13. Indeed, coregulation of IFN-γ and IL-22 in $CD4^+$ T cells by the transmembrane protein class I MHC-restricted T cell-associated molecule (Crtam) was recently demonstrated, thus creating an entry into the mechanisms for coordinately accessing this locus (Yeh et al., 2008).

The IL-10 family is itself an extension of the interferon family, in which IL-28 and IL-29 (as well as the type 1 interferons and IFN-γ) have been identified as additional members (Sheppard et al., 2003). These unusual interferons figure in antiviral responses, but only few studies address their additional functions in innate immunity. As compared to other IFNs, IL-28 and IL-29 bind a distinct receptor made up of the type 1 IFNR1 and the IL10R2, suggesting that novel activities will be mediated.

Two members of the IL-12 family (which also includes IL-12 and IL-35) are present in the roaring '20s, including IL-23, which is critical in Th17 cell maintenance in vivo and in the induction of IL-22; and IL-27, which has been implicated in the induction of IL-10 (Stumhofer et al., 2007). Rounding out the '20s are IL-21, a γc-using IL-2-family member involved in Tfh and Th17 cell function, and IL-25 (structurally a member of the IL-17 family), a potent inducer of IL-4- and IL-13-mediated immunity when administered to mice (Fort et al., 2001). In short, the '20s represent a remarkably potent cytokine decade with great potential to teach us much about immune cell activation and regulation and from which, hopefully, novel therapeutics might be harnessed for inflammatory diseases of humans.

In American history, the lack of regulation in the 1920s resulted in an inevitable financial and social meltdown (encapsulated so well in Fitzgerald's great novel) that resulted in the Great Depression and the hammering out of regulatory networks that survive to this day. Thankfully, evolution has already settled the regulation of the roaring '20s in immunology, and, as revealed in this special issue of *Immunity*, we have begun to glean some hints toward understanding the crossregulation among these diverse and powerful molecules. The comprehensive reviews that follow begin the clarification.

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