Cell

Development in Motion: Helper T Cells at Work

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In mammals, helper T cells orchestrate defense against diverse pathogens. However, these warriors of the immune system can also result in self-inflicted injury culminating in autoimmune and allergic diseases. Recent findings—such as the discovery of the Th17 lineage—have revealed additional complexity in the fates chosen by helper T cells and have begun to reshape our view of how signaling and transcriptional networks generate appropriate and inappropriate immunity.

About two decades ago, a hypothesis was formulated to explain how the varied nature of the mammalian immune response could be linked to the outcome of helper T cell maturation (Mosmann et al., 1986). The initial insights for such diversity came from seminal studies examining antigen-specific helper T cell clones from immunized mice. Based on functional bioassays and protein expression studies, these clones were categorized as Th1 and Th2 cells, which appeared to provide qualitatively different forms of help to B cells and other arms of the immune repertoire by secreting unique subsets of cytokines. Since then, it has been presumed that phagocytic and intracellular defense is orchestrated by Th1 cells, whereas nonphagocytic and extracellular defense is orchestrated by Th2 cells. The price paid for the benefit of adaptive immunity, however, is disease mediated by lymphocytes, including autoimmunity and allergy. In general, it has been thought that Th1 cells are the culprits in organ-specific autoimmunity whereas Th2 cells mediate allergy and asthma.

Recently, a variety of findings have suggested that the spectrum of the immune repertoire could not be completely explained by a simple binary fate choice by a helper T cell (reviewed in Dong, 2006; Weaver et al., 2006). This undercurrent of additional complexity in the Th1-Th2 hypothesis has now culminated in the discovery of a new helper T cell subset (Th17 cells), the characterization of its roles in host defense and autoimmunity, and discovery of a transcription factor, RORγt, that appears to specify its cellular identity (Ivanov et al., 2006). Together, these new findings about how helper T cells function and malfunction have contributed to an emerging portrait of the immune response as a cellular system that exploits key principles of developmentally regulated signaling and transcriptional networks to achieve remarkable plasticity in cell specification. As a consequence of this new information, misfiring in the complex circuitry that leads to immune-mediated disease may now be more amenable to therapeutic intervention.

Mammalian Immune Responses Mobilize Diverse Defense Mechanisms

Adaptive immunity is a cellular network, unique to advanced vertebrate species, which has grown in complexity during evolution, perhaps proportionate to the complexity of microbial enemies that confront terrestrial beings. The cornerstones of adaptive immunity are lymphocytes. B lymphocytes provide defense via the secretion of antibodies. $CD8^+$ (cytotoxic) T cells mediate defense by virtue of their direct killing of infected target cells. In contrast to the other lymphocytes, $CD4^+$ (helper) T cells orchestrate and mobilize a variety of other cell types to do their bidding. Helper T cells activate and/or recruit other lymphocytes, evolutionarily older (innate) immune cells, and even nonimmune cells, such as epithelia, to achieve clearance of the offending pathogen.

A central theme of adaptive immunity is diversity. Each lymphocyte expresses a different antigen receptor to allow a response against virtually any potential microbial invader. To initiate helper T cell differentiation, a migrant dendritic cell enters the lymph node with evidence it has encountered a pathogen in an infected tissue (Figure 1). The rare, patrolling, pathogen-specific T cell, upon encountering the sentinel dendritic cell, must then divide numerous times to achieve a critical mass that is suitable to the defense against the microbial opponent. Because pathogens exhibit diverse lifestyles and evasion strategies, the dendritic cell also instructs the T cell, through both well and poorly understood mechanisms, in the nature and location of the threat. Is it intracellular or extracellular? Is it viral, bacterial, or eukaryotic? In which tissue is it currently residing? A selected helper T cell must, therefore, match the inducible changes in gene expression of its progeny to fit the life cycle of the microbe it is seeking to eliminate. The predominance of helper T cell fate, by virtue of the unique cytokines that are secreted, will determine which other immune and nonimmune cells will be mobilized to battle.

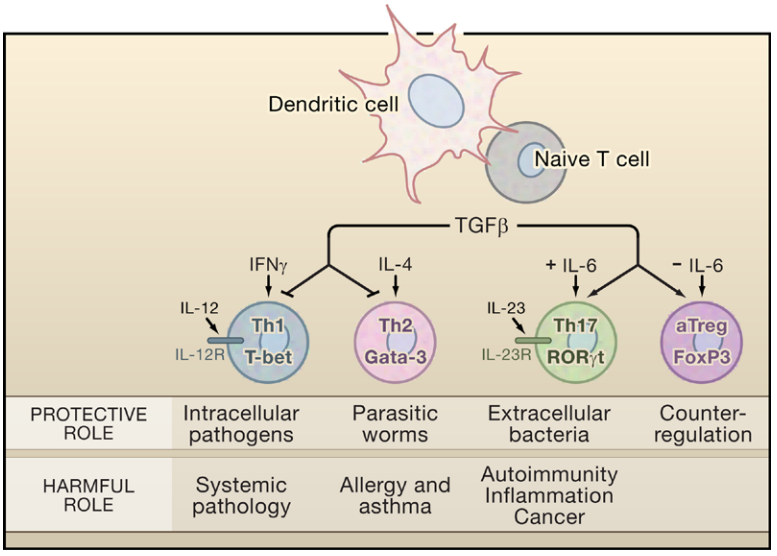

Figure 1. Heterogeneity in Helper T Cell Fates

The helper T cell differentiation process is initiated by signaling from dendritic cell to T cell in the lymph node, resulting in division and differentiation. The mature helper T cells and their signature transcription factors are illustrated. Cytokines (listed above) play a critical role in the induction or repression of the lineages. The different helper T cell subsets have distinct protective and pathological roles (listed below). Host defense is orchestrated by the three major fates, Th1, Th2, and Th17. Adaptive regulatory T (aTreg) cells can downregulate immune responses, although a physiological role in vivo is yet uncertain. The mature helper T cell progeny must eventually exit the lymph node and migrate to infected tissue to exert their function in host defense. Some of the mature progeny may, instead, migrate to B cell follicles to promote antibody subclasses that will suit the particular immune response.

Signaling through the Cell Generations

The signaling by the dendritic cell, which is primarily bestowed on the initial naïve helper T cell (and its first few daughter cells), must somehow be relayed to subsequent cell generations, who are the actual executors of the original instructions. These later cellular progeny of the selected T cell must leave the inductive sanctuary that is the secondary lymphoid organ and migrate to sites of infection and inflammation with a lasting imprint of the inductive signal in order to orchestrate the appropriate defense mechanisms. It may, therefore, not be surprising that epigenetic effects have emerged as a critical principle of gene regulation in the incipient immune response (Ansel et al., 2003; Murphy and Reiner, 2002). Heritable alterations in chromatin modifications and patterns of DNA methylation together with self-reinforcing transcription factor networks provide a mechanism for inductive signals to be remembered through the space and time of cell division and tissue migration.

The Importance of Cytokines

Dendritic cells recognize differences between various classes of pathogens via microbial pattern-recognition receptors and then parlay this information into unique signals to the microbe-specific T cell. Among the most critical signals that direct the induced patterns of gene expression in maturing helper T cells are cytokines (Dong, 2006; Murphy and Reiner, 2002; Weaver et al., 2006). The actions of cytokines that stimulate helper T cell maturation can range from the induction or repression of critical lineage-specifying transcription factors to that of a selective growth factor for a specific lineage (Figure 1). Cytokines are also the critical messengers secreted by mature helper T cells to mediate their influence on other cells during the immune response. IFN-γ is the signature cytokine expressed by Th1 cells, whereas IL-4 secretion is a signature of Th2 cells. IL-17 is a signature of the latest subset to be described, earning it the name Th17. The cytokines produced by a given helper T cell subset often serve as potent inducers of the differentiation of that subset as well as negative regulators of the other subsets.

The Th1-Th2 Paradigm Evolves

One of the most critical cytokines produced by dendritic cells for the induction of Th1 responses is IL-12. This heterodimeric cytokine is typically secreted by activated dendritic cells that have been exposed to bacteria ligands, such as unmethylated CpG and lipopolysaccharide. IL-12 is also secreted by dendritic cells and macrophages in response to parasitization by intracellular prokaryotes, eukaryotes, and some viruses. IL-12 is a key inducer and growth factor of polarized Th1 responses insofar as it represses expression of the transcription factor Gata-3, a key regulator of Th2 commitment. IL-12 also promotes survival, growth, and gene expression of Th1 cells. The IL-12 heterodimer is composed of a p40 and p35 subunit. Experiments antagonizing IL-12 using neutralizing antibodies against p40 or exploiting p40-deficient mice helped to elaborate the important role of IL-12-directed Th1 responses in immunity and autoimmunity.

More than a decade after the initial discovery of IL-12, however, it became apparent that another IL-12 family member, IL-23, shares use of the p40 subunit of IL-12. IL-23 is a heterodimer composed of the nonproprietary p40 subunit and its private subunit, p19. There is a parallel of shared utilization by the receptors for these cytokines. IL-12 and IL-23 receptors both use the receptor IL-12Rβ1. For IL-12, the second subunit is IL-12Rβ2, but for IL-23, the second subunit is IL-23R. Subsequent deconstruction of the pathogenesis of autoimmune disorders, now paying particular attention to the distinction between dual loss of function of IL-12/IL-23 p40 versus single antagonism or deletion of each cytokine alone, led to a startling conclusion: IL-12-driven Th1 cells may not be the major culprits in

organ-specific autoimmunity, but rather a pathogenic, IL-23-driven subset may be to blame (Cua et al., 2003; Langrish et al., 2005; Murphy et al., 2003; Nakae et al., 2003). It was originally thought that the signature cytokines expressed by IL-23-driven T cells were exclusively members of the IL-17 family of cytokines, resulting in the term Th17 cells, but it is now recognized that they also secrete IL-22 (Zheng et al., 2007).

In the short time since the discovery of IL-23, this IL-12-related cytokine and/or the Th17 subset has been implicated in numerous autoimmune and inflammatory conditions including arthritis, multiple sclerosis, psoriasis, and inflammatory bowel disease (Cua et al., 2003; Duerr et al., 2006; Langrish et al., 2005; Murphy et al., 2003; Nakae et al., 2003; Zheng et al., 2007). Most immune mechanisms, however, are likely to serve some protective function and not simply cause damage. The unique contributions to the immune armamentarium provided by Th17 cells appear to be mobilizing acute inflammation/neutrophilic responses and promoting integrity of epithelial surfaces (Dong, 2006; Weaver et al., 2006). IL-17 elicits secretion of attractant chemokines and matrix proteins to yield neutrophil recruitment. In addition, IL-17 and IL-22 have been implicated in barrier function by inducing growth, differentiation, and junctional integrity of epithelia. Thus far, the IL-23-related immune response has been attributed with providing protection against *Klebsiella* and *Citrobacter* bacterial species, tentatively placing the Th17 subset as the defender against extracellular prokaryotes. In retrospect, Th17 cells seem to fill in a gap in the previous repertoire of host defense mechanisms by targeting extracellular pathogens at epithelial surfaces (and perhaps systemically), which primarily require barrier function and/or neutrophil responses.

Lineage Relationships, Inductive Signals, and More Surprises

In the aftermath of the Th17 discovery came a series of elegant studies that have suggested that Th17 cells may not be simple variants of Th1 cells but, rather, a distinct lineage (Harrington et al., 2005; Park et al., 2005). It also quickly became apparent that IL-23, although important in maintaining Th17 responses, might not be the critical inducing cytokine of the Th17 lineage. Instead, it appears that the combination of IL-6 and TGF-β acts in concert to induce Th17 differentiation (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006; Figure 1). Ironically, the Th17-inducing environment suggests a potential kinship between these inflammatory mediators and another subset of helper T cells, called adaptive regulatory T (aTreg) cells, that serve a distinctly anti-inflammatory role. TGF-β, a relatively ubiquitous cytokine, suppresses Th1 (and Th2) differentiation while inducing aTreg formation. IL-6, a prototypical inflammatory cytokine like TNFα and IL-1, thus acts as a switch in the face of TGF-β, directing Th17 cell development by its presence or permitting aTreg cells to develop in its absence (Figure 1).

Master Regulatory Transcription Factors of the Helper T Cell Lineages

After the discovery of cytokine regulation of helper T cell induction, it became apparent that key transcription factors uniquely specify the attributes of each mature helper T cell lineage (Ansel et al., 2003; Murphy et al., 2003; Figure 1). Critical cytokines repress or induce the transcription of the genes encoding these factors. The transcription factors, in turn, define the growth factor preference of the cell by activating private cytokine receptor genes. In addition, the transcription factors function to establish remodeled states of chromatin structure of lineage-specific genes. In essence, the induced expression of these factors, their subsequent stabilization through autocatalytic and feed-forward mechanisms of signaling and transcriptional networks, and their connection to epigenetic remodeling place them as central pieces in the puzzle of how immune response signaling can be imprinted into a heritable cellular identity over the course of division, migration, and time.

Th17 cells do not express the master factors of Th1 and Th2 cells, T-bet and Gata-3, respectively (Harrington et al., 2005; Park et al., 2005; Veldhoen et al., 2006). In addition, expression of the Th1 factor, T-bet, seems to negatively regulate Th17 differentiation. What, then, is the master regulator of Th17 cells? Littman, Cua, and their colleagues suggest that the answer is the orphan nuclear receptor RORγt (Ivanov et al., 2006). Although this transcription factor was not previously implicated in mature T cell function, gene arrays pointed to its unique expression during Th17 but not Th1 differentiation. Using reporter and knockout mice, it was demonstrated that Th17 cells exist constitutively in the intestinal lamina propria and that their development is severely impaired in the absence of RORγt. It was found that RORγt expression is activated by the Th17-inducing cytokine combination of IL-6 plus TGF-β. RORγt also proved to be necessary and sufficient for specifying efficient Th17 induction. Moreover, the loss of RORγt from T cells prevented experimental multiple sclerosis in mice.

These new findings are, thus, harmonious with mounting evidence that Th17 cells represent a distinct developmental lineage. They also support the notion that this subset serves a unique role in the immune repertoire, formerly thought to be the business of Th1 and Th2 cells (Figure 1). They likely act at barrier surfaces to provide integrity and, failing that, local or systemic inflammation to defend us from pathogenic and nonpathogenic extracellular bacteria. In addition, they may well be a major culprit in the T cell-mediated component of numerous autoimmune and inflammatory conditions and could even be implicated in the inflammatory links to cancer (Langowski et al., 2006). From a therapeutic perspective, it did not escape the attention of the authors of the RORγt study that the ligand-binding capacity of a nuclear receptor may render it an easier drug target than its counterpart factors, T-bet and Gata-3 (Ivanov et al., 2006). In the coming months, it is anticipated that many remaining issues about the role of RORγt in Th17

development and function will be addressed. Does RORγt induce the IL-23R in a similar fashion to T-bet inducing IL-12Rβ2? Does RORγt transactivate or induce chromatin remodeling of the genes encoding IL-17 and IL-22? What effect will conditional deletion or antagonism of RORγt have on established immune responses? What will be the precise contribution of RORγt-independent pathways to autoimmunity and antibacterial host defense?

The Future of Development in Motion

The formerly binary choice between Th1 and Th2 cells has now been complicated by the additional choice of the Th17 fate, and further complexity has already been envisioned (Weaver et al., 2006; Zheng et al., 2007). The signaling variables that are integrated at the initiation of the immune response would almost appear to be sensory overload for an environmentally reactive helper T cell, making it difficult to decide among three or more mutually exclusive fates (Figure 1). Given the inherent delay in fate adoption until after the initial cell divisions (Bird et al., 1998), it is possible that a selected helper T cell, instead of making an exclusive choice, might give rise to diverse progeny with tendencies toward differing fates. If a single T cell could apportion an array of lineage-committed precursors, using an ancestral mechanism like asymmetric cell division (Chang et al., 2007), the eventual predominance in fate could be shaped by selective growth and maturation signals specifically tailored to the pathogen. A challenge for the future will be to chronicle the fate of the clonal descendants of a lymphocyte called to battle, in situ. Unveiling the cellular and subcellular details in the initial divisions of a helper T cell in the immune response may provide insight into the unique problems facing developmental signaling networks in cells that are at once dividing, differentiating, and rapidly migrating.

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