Organ-specific specialization of macrophages

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Macrophages in the majority of organs establish residence prior to or just after birth and receive little input from monocytes under homeostatic conditions. Instead, monocytes course through resting tissues, surveying the environment and emigrating through it with minimal differentiation. Under inflammatory conditions, monocytes differentiate to inflammatory macrophages that differ substantially from the resident counterparts. Meanwhile, resident macrophages wane in frequency during inflammation. In the steady state, however, each organ possesses a distinct resident macrophage pool that is remarkably dissimilar from the pool in other organs. Some diverse and general features of macrophages will be reviewed and then a detailed analysis of the peritoneal macrophage population will be presented as a paradigm for unraveling the unique features that a single micro-environment confers. Future directions in the field include gaining a better understanding how, and why, the environment shapes macrophages so distinctly in different organs. What are their specialized, versus common, roles in each locale? In addition, future studies are needed to determine if resident and inflammatory macrophages truly carry out different roles in a given organ.

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The VHL/HIF pathway influences multiple aspects of $CD8^+$ T cell immunity

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During the response to infection and malignancy, $CD8^+$ T cells traffic through a broad range of tissue microenvironments including those with low oxygen tensions, invoking a potential role for the central transcriptional hypoxia response mediated by the von-Hippel-Lindau/Hypoxia-Inducible-Factor (VHL/HIF) pathway. Further, activation of T cells induces accumulation of HIF in normoxia, consistent with a function for HIF in contributing to glycolytic activity during T cell responses. We have found that in the absence of VHL, the primary negative regulator of HIF activity, T cells show enhanced glycolytic metabolism and respond excessively to systemic chronic viral infection, resulting in immunopathology. VHL-deficient CTL are also refractory to chronic antigen-induced immune exhaustion resulting in superior control of viral infection and tumor volume. Loss of VHL alters effector and memory $CD8^+$ T cell differentiation; notably, memory $CD8^+$ T cells develop in spite of sustained glycolytic activity through the contraction and memory phases of the immune response. We also find that hypoxia modulates expression of pivotal transcription factors, effector molecules, and inhibitory receptors in a HIF1α/HIF2α-dependent fashion. Further, deletion of both HIF isoforms in the context of localized tissue infection alters viral clearance and lessens morbidity, suggesting distinct roles for HIF activity in specific contexts. These studies establish a role for HIF-mediated transcription in modulating $CD8^+$ T cell immune responses to both persistent and acute infections and, in turn, have broad relevance in therapeutic strategies to promote viral and tumor clearance.

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How do we make memories and what do they do for us

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The transcription factor FOXO1 functions in multiple aspects of T cell biology—from survival and homing to key steps in commitment to mature effector cells. We present ongoing work directed at understanding how genetic ablation affects function as correlated with chromatin conformation as well a program of gene expression in two distinct examples of CD4 and CD8 T cell differentiation. A conclusion from this work is that FOXO1 behaves as a signal-dependent differentiation factor rather than a lineage commitment factor.

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The intestinal microbiota protects against infection and inflammation induced pathology

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Abstract not to be uploaded on the website

Allergen-specific tissue resident memory CD4$^+$ Th2 cells require IL-2 for differentiation and B cells for maintenance

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Exposure to specific allergens generates Th2 memory CD4$^+$ T cells that contribute to acute, intermittent and recurrent episodes of inflammation associated with asthma. While distinct lineages of Th1 memory cells have been defined based upon functional and migrational properties associated with chemokine receptor expression, it is unknown what lineages of allergen-specific Th2 memory populations can form and how they contribute to airway inflammation. To address this issue, we tracked endogenous CD4$^+$ T cells specific for the house dust mite protein Der p1 after induction of allergic airway inflammation in mice. Intravascular staining in conjunction with tetramer enrichment strategies allowed us to identify and analyze Der p1 specific CD4$^+$ T cells in both the secondary lymphoid organs and lungs throughout all phases of the immune response. Der p1:I-Ab$^+$ cells rapidly expanded in the secondary lymphoid organs and displayed functional characteristics of Th2 cells, including the expression of Th2 cytokines and transcription factors. Der p1:I-Ab$^+$ Th2 cells also accumulated in the lung with successive allergen exposure, reaching equivalent numbers to those found in the secondary lymphoid organs after induction of allergic airway inflammation. After resolution of inflammation, heterogeneous populations of Der p1:I-Ab$^+$ Th2 memory cells were maintained for long periods of time: a primarily CCR7$^+$ central memory-like population in the secondary lymphoid organs and a primarily CCR7$^-$ effector memory-like population that resided in the lung parenchyma. Our studies further demonstrated that the Der p1:I-Ab$^+$ specific cells in the lung are recently described tissue resident memory cells (Trm) and are sufficient to induce asthmatic symptoms when challenged in isolation. To understand how to target these cells with therapeutics, we have dissected the underlying mechanisms of Th2 Trm development and found that their differentiation requires signaling through the IL-2 receptor and surprisingly, their maintenance in the lung requires additional signals provided by B cells.

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UNCOVERING THE INTERPLAY BETWEEN INFLAMMATION AND IMMUNOSUPPRESSION DURING VIRAL PERSISTENCE

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Persistent viral infections are one of the greatest health concerns worldwide and prevention and treatment of these infections represents a major challenge to medicine. Immune escape is required for viral persistence, and many of the suppressive factors involved are being identified. Ultimately, strategies targeting multiple immune factors and effector mechanisms may be required to establish the functionally durable immunity needed for long-term control and/or elimination of persistent infections and then to prevent re-infection. Yet, it is still unclear how prolonged virus replication leads to the expression of the many and mechanistically diverse suppressive factors, pathways, cells and interactions that inhibit immunity to facilitate viral persistence. We recently established a novel link between type I interferon (IFN-I) signaling, immune suppression, and viral persistence. The antiviral activity of IFN-I is well established and critical to maintain virus control throughout many persistent virus infections. However, in addition to its essential and ongoing antiviral function, we discovered that IFN-I signaling is responsible for simultaneously driving many of the immunosuppressive factors, cell types and dysfunctions associated with viral persistence. *In vivo* blockade of IFN-I signaling reversed these immunologic dysfunctions enabling the immune system to control the persistent infection. I will discuss our recent progress on the mechanisms through which IFN-I signaling simultaneously sustains, alters and suppresses antiviral immunity during viral persistence.

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Mechanisms and consequences of inflammasome activation

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Inflammasomes are multiprotein complexes that assemble in the cytosol of cells in response to infectious or noxious stimuli. Once assembled, inflammasomes initiate downstream inflammatory responses by activation of the Caspase-1 protease. Caspase-1 cleaves and thereby activates certain pro-inflammatory cytokines (IL-1 beta and IL-18), and also triggers a rapid and lytic form of cellular suicide called pyroptosis. Several distinct inflammasome complexes have been described, each of which responds to distinct stimuli. Our studies have focused primarily on the NAIP/NLRC4 inflammasomes. These inflammasomes respond to specific bacterial proteins including flagellin and components of bacterial type III secretion systems. We have shown that NAIP proteins are essential for specific recognition of these bacterial ligands, and we have begun to determine the molecular basis for ligand discrimination. We are also interested in understanding the biological consequences of inflammasome activation in vivo. We have developed several novel molecular and genetic tools that are providing insight into the inflammatory pathways downstream of inflammasome activation. This presentation will discuss our latest data on the mechanisms and consequences of inflammasome activation.

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Imaging Treg and Plasma Cell Responses to Infection

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Long lived plasma cells in the bone marrow (BM) are responsible for the production of high serum antibody titers that remain elevated for the lifetime of the host. This population of cells resides in niches within the BM, where numerous factors have been shown to contribute to their maintenance. However, following infection with *Toxoplasma gondii*, the BM environment undergoes significant alterations in composition, including a transient loss of resident plasma cell populations. This decrease in plasma cells correlated closely with the infection-induced loss of Treg populations in the BM. In vivo imaging revealed that BM Tregs can interact directly with plasma cells, and that a high percentage of Tregs and plasma cells interact with a $CD11c^+$ population in the BM. Further analysis of the BM Treg population reveals that these cells display a BM signature that distinguishes them from splenic Tregs. Finally, the depletion of Treg cells in naïve mice results in the loss of plasma cells from the BM. Together, these studies highlight how infection-induced changes in BM populations impact plasma cell homeostasis and suggest that Tregs have a prominent role in this niche.

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Function of miRNA in controlling T cell immunity

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In order to mount a protective response from numerous and enormously diverse microbial pathogens, T cells are able to differentiate into functionally distinct helper T (Th) subsets through acquiring “master transcription factors” in response to different environmental cues. Each Th subset is able to secret selective signature cytokines and to express distinct chemokine receptors that are crucial for proper host defense against foreign invaders. However, such Th responses require adequate control as unrestrained immune responses often lead to overt inflammation and tissue damage. Recently, a class of short regulatory non-coding RNAs, so-called microRNA (miRNA), known for their role in organ development, cellular differentiation, homeostasis, and function, have been demonstrated to be pivotal in immune regulation. Through employing genetic, biochemical, and immunological approaches with whole animal experimentation, we explored the cellular and molecular mechanisms underlying miRNA-mediated post-transcriptional regulation of T cell immunity.

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Links between the cancer genome, the cancer epigenome and immunotherapy design

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Major advances have been made in the immune-based therapy of cancer by antibody blockade of immune inhibitory pathways such as CTLA-4 and PD-1. Anti-PD-1 antibodies have produced objective responses in one third to one half of patients with advanced, chemotherapy refractory melanoma and renal cancer and one quarter of patients with non-small cell lung cancer. Recently, significant response rates to anti-PD-1 antibodies have been shown in bladder cancer, head and neck cancer, gastroesophageal cancer, ovarian cancer and lymphoma. These responses are highly durable, the majority lasting significantly greater than one year and beyond cessation of therapy. Further, expression by tumor cells of ligands for PD-1 is associated with higher response to anti-PD-1 therapy. In exploring the basis for up-regulation of the major PD-1 ligand, PD-L1, on tumor cells, we found that its expression is not constitutive, but rather, is highly associated with lymphocytic infiltration. We identified IFN-γ as an immune signal sensed by the tumor cell that induces PD-L1 expression. In addition to IFN-γ, genes associated with Th1 responses, CTL responses and other inhibitory molecules, such as LAG-3, are up-regulated in lymphocytic infiltrates associated with PD-L1+ tumor cells. These findings indicate that multiple counterbalancing immune effector and inhibitory pathways are operative in the immune microenvironment. They led us to hypothesize a new mechanism of PD-L1 expression in tumors, termed adaptive resistance, distinct from a constitutive mechanism of PD-L1 expression in tumors. The adaptive resistance mechanism implies that other therapies such as vaccines may induce antitumor responses that in turn induce up-regulation of PD-L1. In such a circumstance, vaccination and PD-L1 blockade might produce synergistic anti-tumor activity. We have additionally identified a link between cancer genetics and the immune microenvironment. In human colon cancer, the subset of microsatellite unstable cancers (MSI), which carry a high mutational load, display a potent activated immune infiltrate together with adaptive induction of PD-L1 and multiple other immune checkpoints. Finally, we find that epigenetic modulation alters the tumor microenvironment in a fashion that informs combination therapies with epigenetic modulators and PD-1 pathway blockers.

"How Autoimmunity Gets Started"

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Activation and generation of effector T cells is a lynchpin in various autoimmune syndromes including SLE. It has long been thought that (presumably self-reactive) T cells are important for helping autoreactive B cells to expand, mature and differentiate. However, it has become increasingly clear that autoreactive T cells are involved also in direct tissue damage in organs such as skin and kidney. We have been interested in how such T cells are activated and expanded in vivo during lupus in a murine model. Two major cell types, DCs and B cells, could in principal be important APCs for autoreactive T cells. Their relative roles in initial self-reactive T cell activation, most likely in secondary lymphoid tissues, as well as in expansion and differentiation of such T cells in target tissues are not well defined. Moreover, self-Ags in lupus typically contain endogenous ligands for nucleic acid-specific TLRs. Such TLRs can be expressed by APCs and may govern their activation, which in turn enables them to break self-tolerance in the T cell compartment. We have been taking genetic approaches to try to shed light on these processes in vivo. I will discuss our results from studies in which we have knocked out either B cells, DCs, or key molecules in B cells or DCs in the MRL.Fas^{lpr} murine lupus model.

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Heritable versus non-heritable sources of variation in the human immune system and the nature of the alpha beta TCR repertoire

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In humans there is typically a great deal of heterogeneity in immune measurements between unrelated individuals, and in most cases the basis for this is unknown. Heritable factors are important, but because of continuous interactions with and immune adaptation to non-heritable factors such as commensals and pathogens, we hypothesized that these non-heritable factors might be even more influential in determining the composition of an individual's immune system. To test this hypothesis, we performed a systems-level analysis in 210 healthy twins between 8-82 years of age, focusing on the functional units of immunity, namely the specialized cells and soluble proteins, comprising hundreds of individual measurements. We show that 77% of these are dominated (> 50% of variance explained) and 58% are almost completely determined (> 80% of variance explained) by non-heritable influences. In addition, many of these parameters vary increasingly with age, consistent with environmental exposure with time driving greater divergence. Furthermore in the case of MZ twins discordant for cytomegalovirus, over half of these immune measurements are affected. These results highlight the essentially adaptive nature of the immune system in healthy individuals, likely outweighing all but the most deleterious mutations.

We have also investigated aspects of the human αβ T cell repertoire and find that self-specific T cells are abundant in healthy individuals, although somewhat reduced in frequency in the presence of the cognate antigen. This suggests that the repertoire is broad and comprehensive, with few if any "holes". This is consistent with infectious diseases being the principal evolutionary driver of adaptive immunity.

Viral Triggers of Innate Immunity

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According to current paradigms, antiviral immunity begins when specialized cellular proteins bind viral danger signals that originate from the viral genomes. However, many clinically relevant viruses, including influenza and the respiratory syncytial virus, efficiently block the host antiviral response through virus-encoded proteins that allow the virus to replicate to high titers without intervention of the immune system. The mechanism that mediates the transition from this phase of stealth virus replication to the engagement of the antiviral immune response is unknown.

Our group showed that defective forms of the viral genome that are generated during virus replication are potent danger signals that trigger the host response during infection with a number of respiratory viruses. These defective viral forms have been described in many viruses and were until recently considered an epiphenomenon of growing the viruses *in vitro*. Defective viral genomes stimulate the intracellular pattern recognition receptors RIG-I and MDA5 and maintain their stimulatory ability even in the presence of virus-encoded antagonists. In pioneer work, we showed that natural accumulation of defective viral genomes in the lung during infection with the mouse respiratory pathogen Sendai virus (SeV) or with mouse-adapted influenza A virus correlated with the onset of the antiviral response, and that production of the primary antiviral cytokine IFN beta was limited to the cellular fraction containing defective viral genomes.

Importantly, analysis of respiratory secretions from children infected with the human respiratory syncytial virus demonstrated that detection of defective viral genome is also associated with enhanced expression of transcripts for type I IFNs in humans. Infection of mice with RSV demonstrated that defective viral genomes drastically accelerate the onset of antiviral immunity, reduce virus load, and prevent weight loss and pathology in the lung, suggesting that they have a critical role in determining clinical outcome.

In parallel work, we have identified a specific RNA structure of a SeV defective genome that is essential for the strong stimulatory activity of this molecule. Notably, *in vitro* synthetized oligonucleotides containing this motif show potent immunostimulatory ability *in vivo* demonstrating the potential of these molecules to be used as novel immunostimulants.

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Dendritic cell subsets, T cell subsets and vaccination

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Dendritic cells (DCs) play a key role in the launching of immune responses. We have earlier shown that human Langerhans cells preferentially activate cellular immunity while dermal DC preferentially activate humoral immunity in vitro. In particular, dermal DC induce In vitro the differentiation of naïve CD4+T cells into T follicular helper cells (Tfh). In vivo, Influenza vaccines activate Tfh1 to express ICOS. The frequency of ICOS+Tfh1 directly correlates with the increase of anti-influenza antibody titers. Importantly, these activated Tfh1 cells induce memory B cells to secrete antigen specific B cells. In an effort to understand the interaction of Antigen-Presenting Cells with Vaccines, we exposed purified blood monocytes, IL-4DCs and CD1c blood Dcs to different vaccines. Exposure of DCs to influenza, *Salmonella enterica* and *Staphylococcus aureus*, allowed us to build a modular framework containing 204 pathogen-induced transcript clusters. This framework was then used to characterize the responses of human monocytes, monocyte-derived DCs and blood DC subsets to 13 vaccines. Different vaccines induced distinct transcriptional programs based on pathogen type, adjuvant formulation and APC targeted. Thus, Fluzone® activated monocyte-derived DCs, Pneumovax® preferentially activated monocytes and Gardasil® (HPV) preferentially activated CD1c+ blood DCs. This highlighted the specialization of APCs in response to vaccines. These data might guide the development of improved vaccines.

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Intracellular Nucleic Acid Detection in Host Defense

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Innate immune detection of intracellular nucleic acids is essential for the activation of protective immunity against virus infection. However, a number of sources of self nucleic acids can also activate these same sensors, resulting in autoimmune disease. I will present recent advances in the field of nucleic acid detection, and will discuss our efforts to understand the sensors, their signaling pathways, their antagonism by viruses, and the nature of the endogenous nucleic acids that can trigger autoimmune disease.

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Regulation of Inflammatory gene expression by Long Non-Coding RNAs

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A dynamic and coordinately regulated gene expression program lies at the heart of the inflammatory process (1, 2). This response endows the host with a first line of defense against infection and the capacity to repair and restore damaged tissues. Defined signaling pathways lead to the deployment of transcription factors that collaborate with chromatin and histone modifying complexes to coordinate gene expression in a kinetically defined and cell-type specific manner. Recently, long noncoding RNAs (lncRNA) have emerged as critical regulators of gene expression in diverse biological processes (3). Emerging evidence indicates that immune cells including macrophages express lncRNAs although the role of lncRNAs in controlling gene expression in the immune system remains largely unexplored (4, 5). Here we describe *important* functions of one lincRNA- lincRNA-Eps in controlling immune gene expression. Using recently generated KO mice we elucidate the ability of this lincRNA to restrain the inflammatory response. lincRNA-Eps is transcriptionally repressed in cells exposed to microbial products. RNA-seq profiling of wild type (WT) and lincRNA-Eps KO macrophages reveals lincRNA-Eps as an inhibitor of both basal and inducible immune gene expression. lincRNA-Eps KO animals are hypersusceptible to endotoxin-induced systemic inflammation *in vivo*. LincRNA-Eps mediates these effects by interacting with hnRNPL through a SINE-repeat-element to facilitate RNA-protein complex recruitment to target gene loci to repress gene transcription. Using RNA antisense purification (RAP), ChIP and a series of biochemical approaches we reveal that lincRNA-Eps controls gene transcription by maintaining a repressed chromatin state. Collectively, these studies reveal lincRNA-Eps as a dynamically regulated lincRNA that restrains inducible gene expression associated with host defense.

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Designing immunogen structures and immunization regimens to select specific B cell responses

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One of the major thrusts of our work on HIV vaccine development has been to define immunogens and regimens to induce VRC01-class broadly neutralizing antibodies. In this work we have defined a "reductionist approach" to vaccine design that could be applied to other epitopes on HIV and other pathogens. We will review this line of research, and weigh opportunities and challenges to induce broadly neutralizing antibodies against the VRC01 epitope and other conserved HIV epitopes, in light of recently published structures of the HIV trimeric spike.

Navigating the boundary between Innate and Adaptive Immunity: Cytokines to Stroma

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Pop culture movie star objections notwithstanding, vaccination is arguably one of the most successful medical interventions in human history. While attenuated infectious vaccines induce potent humoral and celluar immune protection, issues related to production costs, shelf life and cold storage, and adverse reactions and reversion to virulence have inspired more recent efforts to be directed toward subunit vaccination. Our lab has focused on identifying and studying the mechanistic underpinnings of vaccine adjuvant formulations that elicit robust cellular responses. These adjuvants produce CD4 and CD8+ T cell responses on par with those observed to many infections challenges. We have identified a number of adjuvant combinations (1, 2) as well as novel formulations (3, 4) that produce cellular responses in both mouse and primates.

Curiously, we have found that some of the signaling pathways and stimuli necessary for producing vaccine-elicited cellular immunity are not the same as those for producing cellular responses following an infection. An example is IL-27, which we recently showed was required for vaccine-elicited, but not infection-initiated, CD4 and CD8 responses (5). In other instances, the use of a reductionist vaccine model system has allowed us to uncover more broadly applicable immunologic mechanisms that were perhaps previously obscured by more complex infectious model systems. Initially using a viral infection model, we observed the well-documented phenomenon of antigen persistence; the maintenance of virally-derived antigens in a host long after (weeks to months) the resolution of productive infection. Previous reports of viral antigen persistence either showed or assumed the participation of follicular dendritic cells (FDC), a stromal cell present within the follicles capable of capturing and holding antigen-antibody complexes for months to years. However, we observed long term antigen (virus or vaccine-derived) persistence in B cell deficient and CR2 deficient hosts, indicating that a cell type other than FDCs must be involved. We eventually identified proliferating Lymphatic Endothelial Cells (LEC) as capturing antigens during productive immune responses, effectively “archiving” them for long periods of time (6). Further we saw that the LECs communicated with hematopoietic APCs, passing off the antigen to be presented to circulating memory T cells, augmenting their rapid protective capacity. These data will be discussed and updated with more recent unpublished data exploring deeper mechanisms of antigen capture by LECs, antigen exchange between LECs and APCs, and corresponding influences on protective immunity.

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Genetics of T follicular helper cell (Tfh) differentiation and function

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T cell help to B cells was one of the earliest discovered functions of T cells, 47 years ago, resulting in the coining of the term 'helper cell'. The vast majority of currently licensed human vaccines work on the basis of long-term protective antibody responses. Generation of long term humoral immunity is a complex process predominantly dependent on germinal centers and CD4 T cell help to B cells. Follicular helper T cells (Tfh) are the specialized CD4 T cells for B cell help. We and others have now resolved many of the stages of Tfh differentiation, and molecules involved. We have also explored human Tfh cell biology and Tfh cell memory. In mechanistic studies of Tfh cells, we have identified novel genetic regulators of Tfh differentiation and function. BCL6 is the defining transcription factor of Tfh cells. However, the functions of BCL6 in Tfh have largely remained unclear. We have defined the BCL6 cistrome in primary human germinal center Tfh cells to assess mechanisms of BCL6 regulation of CD4 T cells, comparing and contrasting BCL6 function in T and B cells. BCL6 primarily acts as a repressor in Tfh cells, and BCL6 binding was associated with control of Tfh cell migration, Tfh differentiation, and repression of alternative cell fates. Interestingly, although some BCL6 bound genes possessed BCL6 DNA binding motifs, more BCL6-bound loci were instead characterized by the presence of DNA motifs for AP1 or STAT. AP1 complexes are key positive downstream mediators of TCR signaling and external stimuli. We show that BCL6 can directly bind AP1, and AP1 and BCL6 co-occupied BCL6 binding sites with AP1 motifs, suggesting that BCL6 subverts AP1 activity. These findings reveal that BCL6 has broad and multifaceted effects on Tfh biology, and provide insight into how this master regulator mediates distinct cell-context dependent phenotypes.(1-4)

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The immunoreceptor TIGIT regulates anti-tumor and anti-viral T cell effector function

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Recent advances in immunotherapy highlight the immune system's ability to respond to many types of cancers. However, tumors constitute highly suppressive microenvironments in which infiltrating T cells are "exhausted" by inhibitory receptors including PD-1, CTLA-4, TIM-3 and LAG-3. We identify TIGIT as an emerging co-inhibitory receptor that critically limits anti-tumor and other $CD8^+$ T cell-dependent chronic immune responses. TIGIT is highly expressed on human and murine tumor-infiltrating T cells. In models of both cancer and chronic viral infection antibody co-blockade of TIGIT and PD-L1 enhanced $CD8^+$ T cell effector function, resulting in significant tumor and viral clearance respectively. This effect was abrogated by blockade of TIGIT's complementary co-stimulatory receptor, CD226, whose dimerization is disrupted upon direct interaction with TIGIT in cis. These results define a key role for TIGIT in inhibiting chronic $CD8^+$ T cell-dependent responses.

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