

CONTROLLING AUTOIMMUNITY: THE BALANCE OF EFFECTOR AND REGULATORY T CELLS

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The immune system exists in a balance between the generation of effector and memory lymphocytes to protect against pathogens and the generation of Foxp3⁺ regulatory T cells (Tregs) to prevent or limit inflammatory reactions. Failure of control mechanisms is the fundamental cause of many inflammatory disorders. We have exploited transgenic mouse models to examine T cell responses to tissue-restricted self antigens and to study how these responses are controlled. Exposure of naive CD4 T cells to tissue antigens leads to the development of pathogenic effector cells, and inflammatory disease. The disease resolves spontaneously, and resolution is associated with the generation and activation of Tregs. Tregs that encounter tissue antigen acquire increased suppressive activity, and a fraction of these Tregs persists as a memory population that continues to control subsequent inflammatory reactions in the tissue. Thus, Tregs go through a sequence of development in the thymus, activation in the periphery to perform their function, and survival as memory cells, which is fundamentally similar to the life history of all lymphocyte populations. The generation and maintenance of pathogenic effector T cells vs protective Tregs are determined by: i) the duration of antigen exposure, with persistent antigen reducing effectors and preserving Tregs, ii) cytokines, especially IL-2, which, at low concentrations, preferentially expands and maintains Tregs, and iii) costimulation, with the appropriate balance of CD28 and CTLA-4 playing a critical role in Treg generation. Elucidating the stimuli that generate and maintain functional Tregs in tissues will likely be valuable for manipulating immune responses in inflammatory diseases and for optimal vaccination and cancer immunotherapy.

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Integrins as force-regulated signaling machines

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Integrins connect the extracellular environment to the cytoskeleton and enable cell migration and organization of cells into tissues. Integrins are targets of clinically approved drugs that block thrombosis and autoimmune diseases such as multiple sclerosis. Integrins have two membrane-spanning subunits and a complex ectodomain organization with 4 or 5 domains in α and 8 domains in β . Integrins are machines that have solved a difficult problem – how to transmit allosteric signals long distances of ~ 200 Å in the extracellular environment. The solution involves three overall conformational states that differ in affinity for ligand by 1,000 to 100,000 fold. The bent-closed and extended-closed conformations are low affinity and the extended-open conformation is high affinity. Integrins relay forces between the actin cytoskeleton and the ligands to which they bind. These forces provide traction for cell migration and act as allosteric effectors to induce and stabilize the high affinity state of integrins. Lateral force exerted by the actin cytoskeleton, such as during retrograde flow of actin, favors hybrid domain swing-out and the open headpiece conformation of integrins. Force times the change in distance between bent and extended-open conformations gives an energy difference that alters the conformational equilibrium that exists in absence of force. Transmission of allostery long distances in the extracellular environment is enabled by application of tensile force, which provides a rigid connection between the ligand binding site and the cytoskeleton. We have begun to test these mechanisms directly in cells, and have evidence from FRET and fluorescence polarization microscopy that when LFA-1 mediates adhesion, the cytoskeleton aligns the ectodomain relative to the direction of cell migration, and applies force to the cytoplasmic domain. Allostery thus can be transmitted through domains that are flexibly connected to one another in the absence of force. Integrins are truly remarkable machines.

Publication pdfs: <http://labs.idi.harvard.edu/springer/pages/publications>

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Probing the relationship between *in vivo* cell dynamics and immune function using intravital imaging

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Immune responses involve cell-cell interactions within lymphoid tissues, trafficking of activated cells to sites of effector function, and the migration of such effector cells within peripheral tissues. To gain a more detailed appreciation of the relationships among cell movement, tissue architecture, and immune function, we have used intravital multiphoton microscopy and a novel multiplex immunohistochemical method to analyze immune cell dynamics and tissue micro-anatomy.

Our data show that migrating T cells follow stromal pathways in lymph nodes, which enhances interactions with dendritic cells. Additional chemokine cues facilitate interactions among rare antigen-presenting and antigen-recognizing cells, with the strength of these interactions as assessed by Ca²⁺ imaging dictating the polarization of the ensuing effector T cell response. In tissue sites, effector cells stop when they perceive antigen and undergo transient activation and polarized cytokine release, followed by tuning of their response to existing antigen levels. Innate immune (neutrophil) responses have been dissected at the molecular level. The role of cell localization in both innate and adaptive immunity has also been addressed using a new method called histo-cytometry that reveals at high resolution the spatial positioning and activation state of cells with complex phenotypes in tissues. These observations show the power of *in situ* imaging in the acquisition of a more accurate picture of the molecular, cellular, spatial, and temporal aspects of cell function and signaling events in host immune responses.

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New Insights on Chemokine Regulation of Cell Migration in Inflammation, Infection, and Autoimmune Disease

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Chemokines and other chemoattractants (such as complement fragments) act via G-protein coupled receptors to direct the movement and activation of leukocytes. Discrete chemoattractants provide tissue-specific signals that localize immune and inflammatory cells to particular compartments. Accordingly, certain receptors for chemokines and chemoattractants may be attractive targets for treating inflammatory diseases without suppressing immunity generally (1). Data regarding the clinical benefit to be derived from the antagonism of such receptors is now available from recent clinical trials that we have performed. These include inhibition of the complement C5a receptor (C5aR) in renal disease as well as the chemokine receptor CCR9 in inflammatory bowel disease, which were each targeted with a unique, receptor-specific, orally-administered small molecule. We have also examined chemokine receptor CCR6 in driving Th17 cells and their associated pathobiologies by using a highly specific inhibitor of CCR6 in several in vivo settings.

In the first case, data from an anti-MPO induced glomerulonephritis model (2) supported testing of a C5aR antagonist (CCX168) to induce disease remission and permit glucocorticoid elimination from the standard of care in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated renal vasculitis. In a double-blind, placebo-controlled trial, dosing of the C5aR inhibitor in flaring ANCA-vasculitis patients showed a marked enhancement of kidney function: reduction of proteinuria and hematuria, improvements in glomerular filtration rates, and increased overall rates of renal remission in the C5aR inhibitor group vs. the standard of care. The data suggested that inhibition of C5aR was effective (and possibly more effective than current standard of care) in treatment of patients with an ANCA-associated renal vasculitis. In the second example, the chemokine receptor CCR9 was inhibited. CCR9 is thought to direct inflammatory T cells to the GI tract during inflammation, including in human Crohn's disease and in ulcerative colitis. In a large Phase II clinical trial of patients with Crohn's disease (the PROTECT-1 trial) inhibition of CCR9 was shown to both induce a clinical response and, importantly, to maintain patients in clinical remission over a prolonged period of therapy (3). The details of the mechanism of action and differences with other clinical studies will be discussed. Finally, CCR6 biology was explored. CCR6 is thought to be important in driving the biology of Th17 cells (4). Among many pathologies, Th17 cells play a central role in human psoriasis and in psoriaform models induced by IL23 and by skin challenge with imiquimod, an activator of TLR 7/8. The mechanism of how CCR6 might be driving the process is not well defined, so we antagonized CCR6 using a small molecule in psoriaform models. In all cases, CCR6 inhibition conferred marked improvement in skin thickening and scaling in the models, both prophylactically and therapeutically. We found that CCR6 in skin was expressed on infiltrating but not resident T cells, and that CCR6 profoundly limited cells that produced IL-17, with concomitant decreases in factors associated with psoriasis such as keratinocyte growth factors. We also found evidence that a unique subpopulation of skin-homing T cells may be prevented from entering the skin when CCR6 is blocked. CCR6 inhibition did not, however, seem to inhibit trafficking of regulatory T cells.

In summary, the discovery of potent and selective small molecule inhibitors of discrete chemokine and chemoattractant receptors, and the use of such inhibitors in both preclinical and clinical settings, has enabled new mechanistic understandings of how cells driven by these receptors are regulated, and paved the way for novel therapies for human disease.

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Regulation of T cell adhesion and migration by Crk family adaptors

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Some Inconvenient New Insights Into TCR Cross-Reactivity and Signaling

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I will discuss our recent results on the inter-related issues of TCR cross-reactivity and signaling.

T cells survey a 'universe' of MHC-presented peptide sequences whose numbers greatly exceed the diversity of the T cell receptor repertoire, leading to speculation that T cell receptors must be highly cross-reactive to ensure effective immunity. Yet, there has been no methodology to exhaustively quantify the cross-reactivity of TCRs via direct experimental means. Estimates of TCR cross-reactivity have generally been derived from theoretical arguments, or extrapolations from small numbers of defined sequences. An extension of this question how the structural manner by which TCRs engage different peptide-MHC ligands influences signaling. Does structure matter? Or is signaling structurally agnostic and simply a byproduct of affinity and oligomerization?

To experimentally quantify TCR cross-reactivity and address the role of cross-reactivity in signaling, we have developed a system to identify MHC-presented peptide ligands for T cell receptors by coupling highly diverse yeast-displayed peptide-MHC libraries with deep sequencing. This system allows for the enrichment and characterization of peptides selected for direct TCR binding without the need for prerequisite knowledge of any ligand. Combining selections and deep sequencing creates a system sensitive enough to discover even incredibly low affinity agonists, rivaling the affinity limits of TCR signaling itself. Our results point to a surprisingly limited degree of TCR cross-reactivity, as well as a role of TCR/pMHC docking geometry in signal initiation.

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Jakinibs Meet Superenhancers: Integrating Cytokine Signaling With Genomic Views of Helper T Cell Specification

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Diverse cytokines induce the growth, differentiation and homeostasis of a wide variety of cells and tissues. Cytokines are also critical for immunoregulation and are important drivers of autoimmune and inflammatory disease. Strong evidence for the importance of cytokines in autoimmunity disease comes from the efficacy of targeted biological therapies. Janus kinases (Jak) are critical elements in signaling via Type I and II cytokine receptors, and the first Jak inhibitors or Jakinibs have now been approved by the FDA. The biological basis of the use of Jakinibs and their mechanism of action in autoimmune disease will be discussed. As a better working understanding of the functional regulation of genome is obtained, cell differentiation is increasingly being viewed from a genomic perspective. How cytokines impact gene expression and the epigenome is an area of intense investigation. How new technologies and new insights into the regulation of genomic responses influence our views of helper T cell specification, lineage commitment and plasticity will also be discussed.

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Controlling the Initiation of TCR Signaling

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Signal transduction by the TCR induces changes in cellular tyrosine phosphorylation by interacting with the cytoplasmic Src family kinases, Lck and Fyn, and with the Zap-70 kinase. The basal activity of the initiator kinase, Lck, is controlled through its interactions with the coreceptors CD4 and CD8 as well as through the opposing actions of the receptor-like protein tyrosine phosphatase CD45 and the cytoplasmic kinase Csk.

Through the use of a system involving a genetically selective small molecule inhibitor of a mutant of the Csk catalytic domain, we have shown that Csk is the predominant means by which Lck activity is negatively in the basal state (Tan et al, 2013). Indeed, our studies suggest that the basal state represents a dynamic equilibrium that is, perhaps, established to set a threshold for activation and established a homeostatic state. Inhibition of Csk rapidly induces the canonical TCR signaling pathway. However, full activation of the TCR signaling pathway requires additional signals that, in thymocytes, can be provided by CD28 costimulatory signals and is likely to involve actin cytoskeletal turnover.

In the basal state, Zap-70 is controlled through intramolecular autoinhibitory allosteric interactions (Yan, et al., 2013). Disruption of autoinhibitory mechanisms by targeted mutagenesis or by mutations in patients results in activation of Zap-70 catalytic activity and can result in autoimmunity in patients. A genetically selective small molecule inhibitor of a Zap-70 mutant reveals the importance of Zap-70 catalytic function in early signaling functions but also revealed the importance of Zap-70 scaffolding function (Au-Yeung, et al., 2010). We have used this genetically selective inhibitor system to explore thresholds of signaling required for peripheral T cell proliferative and effector responses as well as positive and negative selection during thymocyte development. These latter studies reveal unanticipated heterogeneity in the responses of what would appear to be homogeneous TCR transgenic thymocytes.

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How B cells remember

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The acquisition of antibody (Ab) memory is critical for protection from many human infectious diseases and is the basis for most current vaccines. Memory Ab responses are mediated by an expanded memory B cell (MBC) population expressing somatically mutated, isotype-switched IgG B cell receptors (BCRs). In contrast, primary Ab responses involve naïve B cells that express low affinity, unswitched IgM and IgD BCRs. Despite the importance of human B cell memory, the molecular basis of the generation, maintenance and activation of MBCs remains largely unknown. Although one defining feature of MBCs is their expression of IgG BCRs, the advantage conferred by the expression of IgG BCRs on the generation of MBCs or their activation is incompletely understood. Using high-resolution, high-speed, live-cell imaging, we recently determined that as compared to B cells expressing IgM BCRs, IgG BCR-expressing B cells formed signaling active BCR microclusters in response to antigen more efficiently, resulting in enhanced signaling and formation of immunological synapses. We now show that the enhanced signaling of IgG BCRs is dependent on the cytoplasmic tail of the membrane IgG (mIgG) and that the tail contains a highly conserved PDZ binding motif that associates with the PDZ-domain containing synapse-associated protein 97 (SAP97), a member of the membrane-associated guanylate kinase (MAGUK) family. SAP97 and other members of the MAGUK family have been best characterized in neurons, where they play an essential role as synaptic scaffolding proteins in controlling the density, localization, and clustering of glutamate receptors and ion channels at the synapse, and by so doing control excitatory synaptic transmission. We propose a similar role for SAP97 in the IgG BCR immunological synapse and will discuss how SAP97 functions in the generation, maintenance and activation of antigen-specific MBCs.

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Metabolic regulation of T cell function and fate

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Metabolism is the set of biochemical reactions that occur within cells to sustain life. As such, metabolism, by definition, remains the single most fundamental force driving cell fate. Given the critical nature of T cells in clearing and controlling infections and cancer, as well as mediating protective immunity over the long-term, it is logical that a considerable effort is made to target these cells for therapeutic purposes. However, while metabolism regulates the fate and function of T cells, or of any immune cell for that matter, metabolic interventions for manipulating immunity are rare and can be considered a largely untapped opportunity. Our research is focused on establishing fundamental mechanisms of metabolic regulation in T cells, with a view toward identifying new ways to regulate immune cell function through the manipulation of metabolic pathways. Underlying mechanisms of how T cell metabolism and function is altered in the tumor microenvironment will be discussed.

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Adipose innate lymphoid group 2 (ILC2) cells and metabolic homeostasis

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Obesity has increasingly been defined as an inflammatory state associated with infiltration of metabolically active white adipose tissues by classically activated macrophages accompanied by a variety of effector lymphocyte populations. Healthy lean adipose is associated with macrophages that display features of alternative activation, which includes expression of a number of targets of the cytokines IL-4 and IL-13. Using sensitive cytokine reporter mice, we identify ILC2 cells as the source of constitutive cytokines, particularly IL-5 and IL-13, in healthy white adipose tissues that mediate the accumulation of eosinophils and alternatively activated macrophages, or AAMs, in that tissue. Deletion of ILC2 cells results in loss of these normal resident adipose cells; deletion of either AAMs or eosinophils results in loss of metabolic homeostasis as assessed by aberrant adipose accumulation on high-fat diet and by the development of systemic insulin resistance. Activation of ILC2 by cytokines or by systemic helminth infection results in increases in adipose AAMs and eosinophils, and is accompanied by improvements in metabolic homeostasis. Intriguingly, cytokines that expand ILC2 in vivo, such as IL-2 and IL-33, also expand T regulatory cells, particularly in adipose tissues, suggesting important functional interplay between these innate and adaptive cells in the control of metabolic homeostasis.

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Glucose uptake and metabolic programming of T cell subsets

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Lymphocyte activation leads to a rapid transition from a quiescent state to rapid proliferation and differentiation. We have examined this metabolic reprogramming and found that CD4 T cell subsets are metabolically distinct. The effector T cell fates (Th1, Th2, Th17) activate a highly glycolytic program. Regulatory T cells (Treg), in contrast, utilize a more oxidative metabolism and utilize lipids as a major fuel. These metabolic distinctions may allow new understanding and approaches to manipulate immunity. To directly target T cell metabolic pathways we have examined Glut1 regulation and Glut1 conditional knockout animals. Glut1 is a member of the glucose transporter family, of which T cells express several members. Interestingly, normal resting peripheral T cells do not rely on Glut1. Upon activation, however, Glut1-deficient T cells fail to induce glucose uptake and metabolism and do not grow and proliferate. Glut1-deficient Treg, in contrast, appear normal *in vivo*. These data show that activated T cells specifically rely on Glut1 to generate effectors while Treg are capable of utilizing other metabolic pathways. Understanding mechanisms that regulate T cell Glut1 and glucose metabolism, therefore, may provide new tools to modulate immunity the balance of T cell effector and regulatory populations.

Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets.

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mTOR integrates environmental cues to initiate activation and metabolic programs necessary for T cell differentiation and function¹⁻³

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Upon antigen recognition naïve T cells integrate cues from the immune microenvironment to guide T cell activation, differentiation and function. We have been able to demonstrate a critical role for mTOR in integrating these cues in order to guide the outcome of antigen recognition. While current models of T cell differentiation depict the generation of effector cells from a naïve T cell based upon the cytokine environment upon TCR engagement. We propose a new model of T-cell activation, differentiation, and function whereby the outcome of antigen recognition is dictated by mTOR activity and the subsequent up-regulation of selective metabolic function. We propose that upon antigen recognition, based in part on mTOR signaling, naïve T cells are fated to become short term effector cells or long term memory cells. These decisions are facilitated by the upregulation of metabolic machinery necessary to sustain such fates. In the case of CD8+ T cells we provide evidence that this process is guided by the asymmetric partitioning of mTOR activity. We believe that such a model more readily explains the generation of effector and memory cells including the concept of effector and memory Foxp3+ regulatory cells.

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Mechanisms of maternal immune tolerance towards the allogeneic fetus

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How the fetus and placenta avoid rejection by the maternal immune system during pregnancy is a question that has fascinated both reproductive biologists and immunologists alike since the time it was first articulated as a paradox of transplantation immunology nearly 60 years ago. These mechanisms also have major implications for complications of human health, as their disruption has been anticipated to underlie various complications of human pregnancy. Using the mouse as a model organism, our laboratory has identified several key mechanisms that explain the unique immunological status of the fetus and placenta. These include the entrapment of dendritic cells within the decidua, which is the specialized stromal tissue that surrounds the conceptus, and the minimization of decidual macrophage and dendritic cell tissue densities. More recent work has focused on the intrinsic inflammatory characteristics of decidual stromal cells. We have found that these cells engage a developmental program that epigenetically silences the expression of key T cell-attracting chemokines. As a result, activated T cells are not able to accumulate at the maternal-fetal interface to pose a threat to fetal survival. Epigenetic mechanisms also appear to limit the general ability of decidual stromal cells to mount an inflammatory response. These results reveal a novel feature of the maternal-fetal interface within broad implications for the immunology of pregnancy. Supported by grants from the NIH and the American Cancer Society.

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Regulatory T cells occupy an isolated niche in the intestine that is antigen-independent

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CD4+ regulatory T cells (Tregs) maintain immune homeostasis and prevent autoimmunity. T cell receptor-major histocompatibility complex class II signals are necessary for thymic nTreg development and peripheral iTreg generation. However, the requirement for MHCII in Treg homeostasis in tissues such as intestinal lamina propria (LP) is unknown. We examined LP Treg homeostasis in mice in which nTregs develop but lack peripheral TCR-MHCII interactions and no iTregs are generated. nTregs enter the LP and proliferate independently of MHCII in weanlings to fill the compartment. However, new thymic Tregs could access the adult LP and parabiosis showed that Tregs were LP-resident, suggesting a closed niche. This isolated niche was independent of IL-2 but dependent on commensal bacteria. These data demonstrate an LP Treg niche can be filled, isolated, and maintained independently of antigen signals and iTregs. This niche may represent a tissue-specific mechanism to maintain immune tolerance.

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Allogeneic hematopoietic stem cell transplantation as immunotherapy of cancer

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a combination of chemo/radiation therapy, stem cell therapy and cancer immunotherapy for patients with hematological malignancies. Intestinal graft-versus-host disease is a major complication and has a complex pathophysiology with important roles for alloreactive T cells, mucosal immunity, microbial flora and epithelial regeneration. Increasingly, optimization of graft-versus-tumor activity mediated by donor T and NK cells has become the main focus of allo-HSCT.

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Targeting the IL-17 Pathway for the Treatment of Inflammatory Diseases

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Not to be uploaded to website.

Cancer Immunoediting: Mechanistic Insights and Therapeutic Possibilities

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Cancer Immunoediting is the process by which the immune system controls and shapes cancer. We originally envisaged and subsequently showed that, in its most complex form, cancer immunoediting occurs in three phases: Elimination (also known as cancer immunosurveillance, the host protective phase of the process), Equilibrium (the phase in which tumor cells that survive immune elimination remain under immunologic growth control resulting in a state of functional tumor dormancy) and Escape (the phase where clinically apparent tumors emerge because immune sculpting of the tumor cells has produced variants that display either reduced immunogenicity or enhanced immunosuppressive activity) (1-3). Strong experimental data have been obtained using mouse cancer models to demonstrate the existence of each phase of the cancer immunoediting process and compelling clinical data suggests that a similar process also occurs during the evolution of certain types of human cancer. Our efforts now focus on elucidating the molecular and cellular mechanisms that underlie each phase of cancer immunoediting and identifying the critical checkpoints that regulate progression from one phase of the process to the next. We recently used a combination of exome sequencing and epitope prediction algorithms to show that mutant proteins in highly immunogenic tumor cells derived from methylcholanthrene treated immunodeficient mice represent immunodominant, tumor specific antigens for CD8⁺ T cells and that immunoselection is a major mechanism of immunoediting (4). More recently, we asked whether our approach could identify antigens in progressively growing tumors that render them susceptible to checkpoint blockade immunotherapy. T cell lines generated from anti-PD-1 treated mice that had rejected d42m1-T3 progressor sarcoma cells displayed restriction to H-2K^b but not to H-2D^b, suggesting that anti-PD-1 promotes T cell responses to only a limited number of antigens. We then identified expressed nonsynonymous mutations in d42m1-T3 cells using exome sequencing and generated a prioritized list of potential H-2K^b binding epitopes. This analysis predicted two unequivocal “best candidates”—a mutant form of Laminin α subunit 4 (mLama4) and a mutant glucosyltransferase (mAlg8). When tested in vitro, these two epitopes were the only ones among the 61 top predicted H-2K^b binding sequences that stimulated the d42m1-T3 specific T cell lines. These findings were validated by showing that: (i) CTLs expressing TCRs for mLama4 and mAlg8 accumulated over time in d42m1-T3 tumors in anti-PD-1-treated, tumor-bearing mice; (ii) vaccination of naïve WT mice with mutant but not WT forms of Lama4 or Alg8 induced strong CD8⁺ T cell responses; and (iii) naïve mice vaccinated against mLama4 plus mAlg8 controlled outgrowth of d42m1-T3 tumors. These findings reveal that our genomics approach may help identify individuals who would best benefit from checkpoint blockade cancer immunotherapy and may also provide the insights needed to develop personalized cancer vaccines.

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Engineering T Cells to Overcome Tumor Immunosuppression

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Decoding the basic biology of the cellular immune system has permitted advances in the identification and culture of potent effector T cells. Adoptive T cell transfer for cancer and chronic infection is now an emerging field that shows promise in recent trials. Synthetic-biology-based engineering of T lymphocytes to express high-affinity antigen receptors can overcome immune tolerance, which has been a major limitation of previous immunotherapy-based strategies. Advances in cell engineering and culture approaches to enable efficient gene transfer and ex vivo cell expansion have facilitated broader evaluation of this technology, moving adoptive transfer from a “boutique” application to the cusp of a mainstream technology. Recent observations from ongoing clinical trials in patients with leukemia and carcinomas will be discussed. The major scientific challenge currently facing the field is identification of cancer specific targets to avoid on-target, off-tissue toxicity. As the field of adoptive transfer technology matures, the major engineering challenge is the development of automated cell culture systems, so that the approach can extend beyond specialized academic centers and become widely available.

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Clinical activity and biomarkers associated with inhibition of the PD-L1/PD-1 pathway with an engineered Anti-PD-L1 antibody

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Human cancer cells are known to acquire a multitude of genetic aberrations, many of which may be associated with immunogenicity. However, expression of PD-L1 by cancer cells and tumor-infiltrating immune cells may suppress the anti-tumor immune response through the interactions between PD-L1:PD-1 and PD-L1:B7.1. Inhibition of PD-L1 function in cancer patients with the engineered Anti-PD-L1 antibody MPDL3280A as monotherapy has resulted in durable responses across a broad array of human tumor types. The further assessment of biomarkers from pre-treatment and on-treatment tumor specimens from the clinical study of MPDL3280A has resulted in insights into the specific interactions between the host immune response and cancer cells.

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Inflammation and immune suppression in the tumor microenvironment: It's all about RAGE

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Myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAMs) are present in the tumor microenvironment of most patients with cancer and are dominant myeloid cell populations that promote immunoediting, tumor escape, and tumor progression. Because of their immune suppressive activity, these cells are major obstacles to immunotherapy. MDSC and TAMs arise from a common myeloid progenitor cell and are co-opted by the tumor microenvironment to acquire a protumor phenotype. Chronic inflammation plays a major role in recruiting and facilitating these cells, and they synergize with each other to enhance tumor progression. Multiple proinflammatory mediators drive the development of MDSC and TAMs. Since the Damage Associated Molecule Pattern (DAMP) and alarmin, High Mobility Group Box Protein I (HMGB1), is proinflammatory and is a binding partner, inducer, and/or chaperone for many of these proinflammatory molecules, we have examined the effects of HMGB1 on MDSC and TAMs. HMGB1 is in the nucleus of all cells where it binds to DNA. It is also secreted by many myeloid cells and by tumor cells. Secreted HMGB1 binds to multiple receptors on target cells including TLRs 2 and 4, as well as the Receptor for Advanced Glycation Endproducts (RAGE). It is ubiquitously present in the microenvironment of solid tumors where it is produced by MDSC, TAMs, tumor cells, and most likely other cells as well. In vitro experiments demonstrate that neutralization of HMGB1 deters the differentiation of MDSC and TAMs from bone marrow progenitor cells, reduces the T cell suppressive potency of MDSC, and limits MDSC-macrophage cross-talk. Treatment of tumor-bearing mice with either antibodies or inhibitors that neutralize HMGB1 delays tumor progression, reduces levels of tumor-infiltrating MDSC, and restores T cell homing to lymph nodes. These studies suggest that HMGB1 regulates tumor progression by impacting MDSC. Since HMGB1 regulates, facilitates, and enhances the action of many of the proinflammatory mediators that drive MDSC, HMGB1 may be a key molecule that controls this immune suppressive cell population.

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