

The Cancer Research Institute 2013 Annual Symposium: Dynamics of Host–Tumor Interaction

Abstract

The 21st annual Cancer Research Institute (CRI) cancer immunotherapy symposium, entitled "Dynamics of Host–Tumor Interaction," was held in New York City from September 30 through October 2, 2013. The symposium comprised 27 presentations, organized into five sessions and exploring such topics as the role of chronic inflammation in creating a protumorigenic microenvironment, the interactions between the cancer stroma and immune cells in trafficking and cancer metastasis, the role of the host microbiota in immune responses to cancer, and the interactions between cancer cells and immunoregulatory elements, including regulatory T cells and T-cell checkpoint proteins. The conference began with a keynote address by Michael Karin, recipient of the 2013 Coley Award, who discussed the role of inflammation as a Janus-faced process in the body's fight against cancer—both tumor destroying and tumor promoting. The conference concluded with a session on therapeutics and translational research aimed at improving existing cancer immunotherapies. *Cancer Immunol Res*; 2(2); 105–11. ©2014 AACR.

Introduction

In their 2011 review, "Hallmarks of Cancer: The Next Generation," Hanahan and Weinberg argued that "the biology of tumors can no longer be understood simply by enumerating the traits of the cancer cells but instead must encompass the contributions of the 'tumor microenvironment' to tumorigenesis" (1). This recognition by cancer biologists of the importance of the tumor microenvironment reflects many years of work by tumor immunologists, who have long been interested in host factors that promote or combat tumor development. Recent years have seen an increased focus of research on the tumor microenvironment among both cancer biologists and tumor immunologists interested in improving the outcomes of cancer immunotherapies (2).

Despite recent advances, such as the U.S. Food and Drug Administration approval of sipuleucel-T (Provenge; Dendreon) and ipilimumab (Yervoy; Bristol-Myers Squibb) for prostate cancer and melanoma, respectively, it has become clear that interactions between the host immune system and the tumor microenvironment likely limit the effectiveness of immunotherapies. A better understanding of host–tumor interactions is therefore needed to improve the outcome of immunotherapy for cancer.

To address this need, the Cancer Research Institute (CRI) convened its 21st annual cancer immunotherapy symposium, entitled "Dynamics of Host–Tumor Interaction," which was held in New York City from September 30 through October 2, 2013. The five sessions covered topics such as the role of chronic inflammation in creating a protumorigenic microenvironment, the interactions between the cancer stroma and immune cells in trafficking and cancer metastasis, the role of the host microbiota in the immune response to cancer, and

the interactions between cancer cells and immunoregulatory elements, including regulatory T cells (Treg) and T-cell checkpoint proteins.

The symposium began with the inaugural William B. Coley lecture, delivered by Michael Karin, recipient of the 2013 Coley Award, on the molecular mechanisms underlying inflammation-induced tumorigenesis in colon cancer. Karin's lecture has been summarized in the Milestones in Cancer Immunology feature in the December issue of this journal (3). Here, we summarize the research presented in the five sessions.

Session 1: Tumor Microenvironment

The particular outcome of an immune response to cancer—eradication, control, or promotion—depends in part on the recruitment and migration of immune cells to the site of tumor development and the subsequent interactions among these cells. These processes are not completely understood. One challenge has been that most studies to date have used technology that has allowed only static snapshots of histology or relied upon survival time intervals to infer the occurrence of past cellular events. The six speakers in this session used new methodologies to explore real-time dynamics of immune cell movements in living animals. Multi-photon intravital microscopy and spinning-disc confocal microscopy have allowed investigators to peer into living tissues, such as lymph nodes, and watch as different types of immune cells, illuminated by fluorescence, migrate to their destinations and interact with other immune cells, cancer cells, and the tumor stroma.

CRI investigator Alex Huang of Case Western Reserve University School of Medicine (Cleveland, OH) opened the session with a discussion of how his laboratory has used intravital two-photon microscopy to trace the traffic patterns of CD4⁺ versus CD8⁺ T cells in the lymph node. Huang and colleagues have found that CD4⁺ T cells enter and traverse a lymph node much

doi: 10.1158/2326-6066.CIR-13-0225

©2014 American Association for Cancer Research.

faster than CD8⁺ T cells and differ in the amount of time each spends interacting with MHC molecules on the surface of dendritic cells (4). They have used this technique to examine the behavior of antigen-presenting cells (APC) in the brain, and they discovered that CX3CR1⁺ APCs are able to extend their cellular processes across the blood–brain barrier for short intervals into the vascular lumen (5).

Thorsten Mempel of the Massachusetts General Hospital (Boston, MA) used the same microscopy technique to track the migration of both Treg and effector T cells (cytotoxic T lymphocytes; CTL), and to explore their interactions at the single-cell level (6). The goal is to identify the different T-cell receptor–dependent signaling pathways activated in Treg and CTL in response to binding of their cognate antigens, in the hopes that such differences will inform the design of therapies that can selectively enhance CTL function while attenuating Treg function to favor tumor rejection.

Zena Werb of the University of California, San Francisco (San Francisco, CA), used spinning-disc confocal microscopy to study changes in the extracellular microenvironment, including modifications to collagen synthesis and deposition that occur as breast carcinoma progresses. Werb and colleagues found that the transcription factor GATA3, whose expression is lost in many breast cancers, induces the expression of microRNA miR-29b, which inhibits various prometastatic factors involved in angiogenesis, collagen remodeling, and proteolysis (7). The GATA3–miR-29b axis is therefore a potential target of therapeutic intervention.

Retrospective studies across many different types of cancers have correlated the presence of tumor-infiltrating lymphocytes (TIL) with better prognosis (8, 9). However, factors that limit the migration of lymphocytes into tumors are largely unknown. CRI investigator Shannon Turley of the Dana-Farber Cancer Institute (Boston, MA) showed that T cells can migrate through a lymph node on a conduit system comprising different substrates that include fibroblastic reticular cells (FRC). Turley showed that FRC ablation with diphtheria toxin results in reduced numbers of T cells and antigen-specific T-cell responses. A thick layer of collagen fibers surrounds the corona of many solid tumors. Turley showed that T cells are preferentially attracted to the conduit system provided by the collagen fibers on the tumor periphery and are thus prevented from entering the tumor core. Antifibrotic drugs such as pirfenidone can reduce the collagen-rich tumor corona, allowing increased migration of CD8⁺ TILs into the tumor core. Pirfenidone has no antitumor effect in RAG^{-/-} mice, indicating that the antitumor activity is mediated by adaptive immune cells.

Tomasz Zal of The University of Texas MD Anderson Cancer Center (Houston, TX) analyzed aspects of the tumor microenvironment that may affect immunotherapy with checkpoint antibodies (10). Zal used iDISC-enhanced intravital microscopy to visualize the spatiotemporal dynamics of immune response in a mouse model of pulmonary metastases. Triple fluorescence–labeled endogenous subsets of Treg, T cells, and dendritic cells as well as introduced cyan fluorescing-MCA fibrosarcoma tumor cells were used to

show that CD11c phagocytic dendritic cells are the first immune cells to arrive in metastases, followed by FoxP3⁺ CD4 Treg and then T effector cells. Treg accumulate to a higher degree than T effector cells, thereby inhibiting an effective antitumor response. These researchers showed that the T cells are attracted to and retained at the metastases by the tumor-associated CD11c phagocytic dendritic cells, whose subset expressed high levels of PD-1 ligands, and not by the tumor cells. They found that MCA lung lesions were efficiently cleared after treatment with anti-PD-L1, which increased intratumoral CD8 T-cell/Treg ratios, but not with anti-PD-1 (RMP1-14), even though the tumor cells have minimal expression of PD-1 ligands. In contrast, anti-PD-L1 therapy failed to clear pulmonary metastases of syngeneic B16 melanoma that constitutively expressed PD-L1 ligands but recruited much fewer CD11c dendritic cells. Their studies highlighted the importance of intratumoral phagocytic dendritic cells for antitumor immune responses.

CRI researcher Khashayarsha Khazaie of Northwestern University presented work that he and Fotini Gounari of the University of Chicago have conducted showing that the density and type of T lymphocytes present within colon tumors dictate the clinical outcome of colon cancer. They propose that the key factor is whether or not the immune cells are proinflammatory. While high densities of Th17 cells promote inflammation, Treg suppress inflammation. However, these investigators have found that Treg are frequently converted into inflammation-promoting T cells in colon tumors by the activation of Wnt/β-catenin–RORγt signaling, raising the possibility that inhibitors of RORγt signaling might be effective in treating colon cancer (11).

Session 2: Metabolism in the Microenvironment

Tumor development and immune cell activation share certain metabolic similarities, as both processes require increased nutrients and biosynthesis for cell growth and proliferation. Recent studies have confirmed that both tumorigenesis and T-cell activation utilize aerobic glycolysis, which supports the rapid production of ATP for cellular function and provides intermediate metabolites that can be used as carbon sources for biosynthesis and proliferation. The shared pathways and mechanisms allow knowledge gained from studies in one system to facilitate the understanding of the other. However, there are also differences between T-cell activation–induced metabolic reprogramming and the cellular metabolic processes hijacked by a developing tumor. Understanding the similarities and differences in both systems is therefore necessary to inform the design of novel cancer therapies.

CRI investigator Jeffrey Rathmell of Duke University (Durham, NC) presented work on glucose metabolism in T-cell activation. The differentiation and specification of T-cell subsets are determined by the initial activation signals and the cytokines in the local environment. These researchers used a mouse model with a conditional knockout of glucose transporter 1 (GLUT1) to examine the metabolic reprogramming during T-cell activation. Fourteen members of the glucose transporter family have been identified. Naïve, resting peripheral T cells utilize various glucose transporters (GLUT1,

GLUT3, GLUT6, and GLUT8). However, upon activation with interleukin (IL)-2, inflammatory effector T cells (Th1, Th2, and Th17) switch to the preferential use of GLUT1 for increased glycolysis similar to that of cancer cells, whereas Treg continue the lipid-oxidation metabolism independent of GLUT1. GLUT1-deficient T cells do not grow or proliferate. In fact, GLUT1-deficient T effector cells undergo apoptosis while GLUT1-deficient Treg appear normal. These results are corroborated by the requirement of functional GLUT1 in T effector cells for the induction of various inflammatory diseases (12).

Nicholas Restifo of the National Cancer Institute (NCI, Bethesda, MD) described work on TIL-based adoptive cell transfer (ACT) immunotherapies in mice and humans. Along with his colleagues, Restifo found that the antitumor responses in melanoma are associated with the ACT of less differentiated and more stem-like T cells with longer telomeres. He discussed the use of metabolic profiling to select the less differentiated and thus more effective T cells for ACT-based immunotherapy for patients with metastatic cancer. Restifo described efforts in identifying targets in the metabolic pathways to improve immunotherapy. Upon antigen encounter, naïve CD8⁺ T cells rapidly switch from fatty acids oxidation metabolism to glycolysis to sustain effector function and terminal differentiation. Using fluorescent glucose analog 2 NBDG, they quantified glucose uptake in activated CD8⁺ T cells and found that cells that have limited glucose incorporation have a molecular profile characteristic of memory precursor cells with an increased capacity to enter the memory pool compared with those with high intake of glucose. They showed that activation of CD8⁺ T cells in the presence of the glycolysis inhibitor 2-deoxyglucose increased T-cell survival and enhanced the generation of CD8⁺ T memory cells and their antitumor function. Results from this investigation indicate that pharmacologic targeting of metabolic pathways during T-cell priming can promote the generation of long-lived CD8⁺ T cell immunity (13).

Research in Erika Pearce's laboratory at the Washington University School of Medicine (St. Louis, MO) focuses on dissecting the mechanisms of metabolic regulation in T cells to identify new ways of manipulating the function of immune cells particularly in the tumor microenvironment. While ATP can be generated from both aerobic glycolysis and oxidative phosphorylation (OXPHOS), Pearce and colleagues showed that mitochondrial ATP from OXPHOS is required for the activation of naïve T cells. Activated T cells switch from OXPHOS to aerobic glycolysis not for survival and proliferation but for optimal cytokine production and effector function. When glycolysis is blocked, activated T cells lose their ability to produce IFN γ ; this defect is translational and is imposed by the binding of the glycolysis enzyme GAPDH to the 3' UTR of the IFN γ mRNA. Therefore, T-cell exhaustion is linked to metabolism, not exposure to antigens; activated T cells use aerobic glycolysis to remove the GAPDH inhibition of IFN γ production and effector function. These results implicate aerobic glycolysis as a target to control T-cell effector functions (14).

Session 3: Innate Immunity

Over a century ago William Coley proposed that killed bacteria might be used to treat cancer, and even suggested that cancer might have an infectious etiology (15, 16). In the past decade, bacteria have (re)emerged as a focus of cancer research, not as a primary cause of cellular transformation, but as a cause of chronic inflammation that may promote tumor progression, particularly in colon and other mucosal cancers.

Through deep-sequencing metagenomic analyses, Wendy Garrett of the Harvard School of Public Health (Boston, MA) and colleagues found that fusobacteria are associated with human colorectal adenomas and carcinomas; stool samples from patients with these cancer types are enriched in fusobacteria. Using a mouse model of intestinal tumorigenesis, Garrett and colleagues found that *Fusobacterium nucleatum* promotes a protumorigenic immune response within the tumors (17). They showed that fusobacteria are associated with altered composition of immune cells in the tumor microenvironment; specifically, tumors from *F. nucleatum*-exposed mice are enriched in tumor-associated macrophages and myeloid-derived suppressor cells. These results suggest that fusobacteria are involved in both the early stages of tumorigenesis and the progression of colorectal cancer, leading her to argue that "the microbiota may prove as influential as stromal cells and immune cells in the tumor microenvironment" (18).

Romina Goldszmid of the NCI showed that response to both CpG immunotherapy and platinum chemotherapy were deficient in antibiotic-treated or germ-free mice. In both cases, innate myeloid cells were responsible for the effect. In the case of CpG immunotherapy, monocyte-derived cells in the tumor microenvironment failed to produce TNF and interleukin (IL)-12, whereas the deficient response to chemotherapy was correlated with reduced production of reactive oxygen species by tumor infiltrating myeloid cells. These results indicate that an intact commensal microbiota is necessary for an optimal response to immunotherapy and chemotherapy (19).

Richard Blumberg of the Brigham and Women's Hospital (Boston, MA) and colleagues investigated the role of FcRn (neonatal Fc receptor for IgG) in regulating CD8⁺ T-cell responses against colorectal cancer. FcRn is a bidirectional IgG transport receptor, found in endothelial, epithelial, and hematopoietic cells, that is essential for protection against mucosal pathogens. FcRn is known to play an important role in cross-presentation of IgG-complexed antigens to CD8⁺ T cells by dendritic cells (20). Furthermore, FcRn-knockout mice have more colon tumors and FcRn⁺ dendritic cells correlate with survival in patients with colorectal cancer. Blumberg and colleagues have now shown that FcRn-mediated protection against colorectal cancers and lung metastases is driven by dendritic cell activation of tumor-reactive CD8⁺ T cells via cross-presentation of IgG-complexed antigens, thus suggesting that FcRn plays a role in priming antitumor immune surveillance.

CRI investigator Joseph Sun of Memorial Sloan-Kettering (New York, NY) and his colleagues are studying the role of recombination activating genes (RAG) in natural killer (NK) cells—innate immune cells that protect against virus-infected and cancerous cells. The expression of RAGs is required for the

development of B and T cells, but not for the development of NK cells, as evidenced by the fact that RAG-deficient mice have normal numbers of NK cells (21). However, these investigators have shown that NK cells from RAG-deficient mice are hyper-responsive, and RAG-deficient mice are more resistant to tumor challenge. Using fate-mapping mice to study RAG expression in wild-type NK cells, they found that a significant proportion of NK cells showed evidence of having expressed RAG genes. NK cells from RAG-deficient mice are prone to apoptosis, particularly following virus-driven proliferation. Sun and colleagues propose that RAG expression might endow NK cells with cellular "fitness" during rapid cell proliferation or cellular stress, and they are now investigating the mechanism that promotes the hyper-responsiveness of RAG-deficient NK cells.

Jack Bui, a former CRI postdoctoral fellow and now a professor at the University of California, San Diego School of Medicine (La Jolla, CA), presented work from his laboratory on factors that modulate tumor immunogenicity (22). Using microarray analysis, Bui and colleagues found that IL-17D is expressed in highly immunogenic "regressor" tumor cells but not in poorly immunogenic "progressor" tumor cells. They showed that enforced overexpression of IL-17D in some progressor tumor cells is sufficient to induce the rejection or growth delay of these tumor cells; the mechanism of action of IL-17D is to stimulate the production of monocyte chemoattractant protein-1 (MCP-1 or CCL2), which recruits NK cells to the tumor. The recruited NK cells could kill cancer cells directly, but another contribution of NK cells to the IL-17D-mediated tumor rejection is to secrete cytokines that attract other immune cells and lead to M1 macrophage development and the activation of antitumor adaptive immune responses. This research raises the possibility that IL-17D could be used therapeutically to recruit NK cells to poorly immunogenic tumors.

Thaddeus Stappenbeck of the Washington University School of Medicine (St. Louis, MO) studies stem cell replacement and organization in tissue regeneration. Stappenbeck and colleagues used the intestinal crypt as a model system to explore factors involved in tissue repair and regeneration. They found that the noncanonical Wnt ligand Wnt5a was required for crypt regeneration. They delineated the role of Wnt5a in crypt formation: Wnt5a inhibits the proliferation of intestinal epithelial stem/progenitor cells and potentiates TGF- β signaling to promote the formation of new crypt units for tissue repair (23). Understanding the molecular mechanisms of tissue repair may provide useful lessons for understanding cancer since wound healing and tumor stroma formation share multiple features (e.g., inflammation, fibrin deposition, and angiogenesis) and tumors may be thought of as "wounds that do not heal" (24).

CRI-supported researcher Irving Weissman of Stanford University School of Medicine (Stanford, CA) presented work on CD47, which he dubbed the cancer "don't eat me" signal. In the late 1990s, the Weissman laboratory discovered that mouse cancer cells often upregulate CD47 on their cell surface. CD47 is a ligand for macrophage receptor SIRP α . Antibodies that block CD47 enable tumor cells to be phagocytosed by mouse

and human macrophages *in vitro* (25). Using tumor models of xenograft human cancers growing in immunodeficient mice, they showed that several weeks of infusion with blocking antibodies to CD47 shrunk primary tumors and eliminated metastases. In recent work, investigators from the Weissman laboratory showed that anti-CD47 antibody-mediated phagocytosis of cancer cells by macrophages primes an effective antitumor T-cell response (26). They continue to explore how the CD47-mediated avoidance of programmed cell death and programmed cell removal by precancerous stem cells can alter the dynamics of incipient tumor formation to inform the development of anti-CD47 blocking antibodies for cancer therapy.

Session 4: Highlights from the Abstracts

Of the 108 posters displayed at the 2013 CRI symposium, five were chosen for oral presentations. Michele Ardolino, a CRI postdoctoral fellow in David Raulet's laboratory at the University of California, Berkeley, presented research that helps to explain why NK cells fail to efficiently eliminate tumor cells with low MHC expression. According to the "missing self" hypothesis proposed by Kärre and colleagues (27), NK cells have evolved the ability to kill cells that lack or express low levels of MHC, such as virally infected cells and some tumor cells; the lack of efficient killing *in vivo* is therefore perplexing. Ardolino and colleagues showed that even a small fraction of MHC-deficient tumor cells can render NK cells hyporesponsive (anergic). They found that treatment of mice harboring MHC-deficient tumors with NK cell-activating cytokines such as IL-18 and IL-12 restores NK cell responsiveness, suggesting a potential role for cytokine-based immunotherapy in patients with MHC-deficient tumors.

Using a genetically engineered mouse model of pancreatic ductal adenocarcinoma, Katelyn Byrne of the Perelman School of Medicine at the University of Pennsylvania (Philadelphia, PA) found that the combined regimen of chemotherapy (gemcitabine with nanoparticle albumin-bound paclitaxel, Abraxane; Gem/Abrx) plus agonist CD40 monoclonal antibody (FGK45) induced tumor regressions in more than 50% of mice, whereas treatment with either therapy alone induced only rare regressions. CD40 is a costimulatory protein on APCs that is required for their activation. The combined Gem/Abrx/FGK45 treatment increased the ratio of CD4⁺ T helper or CD8⁺ T effector cells to regulatory T cells in tumors. Byrne argued that these results support the hypothesis that CD40 agonist plus chemotherapy can serve as an effective vaccine for this otherwise immunosuppressive tumor.

Margaret Callahan, a CRI Lloyd J. Old Fellow at Memorial Sloan-Kettering Cancer Center, presented results from pharmacodynamic studies of patients treated with a combination of ipilimumab and nivolumab. Previous results indicated that tumors expressing PD-L1 show a greater response to nivolumab treatment, providing a rationale for targeting this treatment to patients with PD-L1-expressing tumors (28). However, Callahan and colleagues found that tumor expression of PD-L1 was not predictive of clinical

outcome in patients treated with ipilimumab plus nivolumab, suggesting that the combination treatment may overcome the limitations of each monotherapy.

Greg Delgoffe of St. Jude Children's Research Hospital (Memphis, TN) showed that the receptor neuropilin-1 (Nrp1) expressed by Treg is necessary to suppress antitumor immunity but not autoimmunity. Immune cell-expressed ligand semaphorin-4a (Sema4a) binds Nrp1 and stabilizes Treg; disruption of the Sema4a:Nrp1 pathway in wild-type mice can increase antitumor immunity. These results raise the possibility that signaling through the Nrp1:Sema4a pathway could be a target for immunotherapy.

Corrie Painter, a CRI postdoctoral fellow at the University of Massachusetts Medical School (Worcester, MA) described efforts to establish the zebrafish (*Danio rerio*) as a model system for studying adaptive immune responses to melanoma. Painter showed that genes in the adaptive immune system are conserved between zebrafish and humans. Using zebrafish genetically engineered to produce tumors that resemble human melanomas, she has generated transgenic lines with fluorescence-labeled T cells to track the movements of T cells within the intact tumor microenvironment. An advantage of this approach is that zebrafish are transparent, so one can look right into their tissues and observe growing tumor using wide-field fluorescence microscopy. Preliminary results indicate that lymphocytes initially are able to penetrate an early melanocytic lesion but get pushed to the periphery as the tumor progresses.

Session 5: Therapeutics

The last session of the symposium was devoted to clinical and translational research relevant to three active areas of immunotherapy: adoptive T-cell transfer, checkpoint blockade therapy, and cancer vaccines. The first presentation was given by CRI investigator Peter Savage of the University of Chicago on the biology of Treg. Treg have emerged as an intense area of research in recent years, along with the recognition of the importance of negative regulators of the immune response in both autoimmunity and cancer. Work in Savage's laboratory is devoted to answering basic questions about Treg biology, including where Treg develop, what antigens they recognize, and what role they play in modulating cancer development and metastasis. By tracking a single specificity of Treg identified in a mouse model of prostate cancer, Savage and colleagues showed that these Treg develop in the thymus and are then recruited to tumor sites rather than being induced to develop from CD4 precursors in the tumor environment. They also showed that these Treg recognize a prostate-associated antigen rather than a cancer-specific antigen, and that the thymic development of Treg is dependent on the transcriptional regulator Aire. Aire was originally identified as the gene responsible for the human disease APS-1 (autoimmune polyendocrine syndrome 1); loss-of-function mutations in Aire result in widespread organ-specific autoimmunity, suggesting that Aire is involved in peripheral tolerance. Subsequent research showed that Aire induces the expression of an "immunologic self

shadow" in the thymus; these antigens allow for negative selection of T cells reactive to peripheral organ-specific antigens (29). These authors propose that Aire has two main functions: negative selection of self-reactive T-cell clones and development of organ-specific Treg (30).

For a variety of immunotherapies, including adoptive T-cell transfer and vaccination, T-cell tolerance is a major challenge. Many tumor-associated antigens that could promote T-cell cytotoxicity are also self-antigens; the immune system has ways of defusing such self-reactive T cells to prevent autoimmunity, for example, through deletion or induction of functional anergy. CRI investigator Ryan Teague of Saint Louis University School of Medicine (St. Louis, MO) and colleagues showed previously that combined checkpoint blockade therapy with antibodies to CTLA-4, PD-1, and LAG-3 could prevent deletion of adoptively transferred leukemia-reactive CD8⁺ T cells in a mouse model of T-cell tolerance (31). In more recent work using mouse models, the Teague laboratory found that T-box transcription factor T-bet was required to overcome the tolerizing signals *in vivo*. T-cell tolerance corresponds to low expression of T-bet; the effector T cells rescued with the combined checkpoint blockade have restored levels of T-bet expression.

Checkpoint blockade with anti-CTLA-4 antibodies has emerged as an effective treatment for metastatic melanoma and potentially other cancers. Many questions remain, however, about the mechanism of anti-CTLA-4 action. Sergio Quezada, a former CRI postdoctoral fellow and now a CRI investigator at the University College London Cancer Institute (London, UK), had previously shown that anti-CTLA-4 antibodies affect the balance between CTL and Treg. Many tumors have more Treg than T effector cells (Teff). Quezada showed that anti-CTLA-4 treatment results in the selective depletion of tumor-infiltrating Treg, thus reversing the balance between Teff and Treg and altering the immunosuppressive tumor microenvironment. The preferential depletion of tumor-infiltrating Treg is mediated by antibody-dependent cell-mediated cytotoxicity and is completely dependent on the expression of FcγRIV by tumor-infiltrating macrophages, which preferentially target Treg over Teff because Treg express higher levels of CTLA-4 (32).

Mario Sznol of the Yale Cancer Center (New Haven, CT) summarized results of recent clinical trials with checkpoint blockade antibodies. In a phase I study of anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) in patients with metastatic melanoma, the objective response rate for the 52 evaluable patients was 40%, with an even higher response rate among patients enrolled in cohorts above the lowest nivolumab dose level (33). Sznol believes that in most cases antigen-specific immunization is not necessary to achieve a response to immunotherapy, and that autoimmunity is likely an unavoidable consequence of checkpoint blockade therapy in subsets of patients. Many autoimmune toxicities such as gastrointestinal and lung toxicity might be alleviated or reduced with supportive treatments, such as granulocyte macrophage colony-stimulating factor (GM-CSF), steroids, anti-TNF, and mycophenolate. Sznol described numerous encouraging results in patients with very advanced refractory

disease, of multiple malignancies, who were treated on checkpoint inhibitor trials of ipilimumab, nivolumab, MK-3475 (anti-PD-1), MPDL3280 (anti-PD-L1) and the combination of ipilimumab and nivolumab.

Jay Berzofsky of the NCI discussed the "push-pull approach" that his laboratory uses to optimize vaccine formulation and delivery. They begin with epitope enhancement, modifying the epitope sequence to increase binding affinity for MHC molecules. The next step involves incorporating adjuvants, such as TLR ligands, cytokines, and other agonists to "push" the immune response along and improve both the quality and the quantity of these responses. The last step is blocking negative regulators of the immune response to "pull" it further. The Berzofsky laboratory has identified a role for anti-TGF- β antibodies in blocking the negative regulation by type II NKT cells (as well as Treg); they tested a human monoclonal antibody to TGF- β in patients with malignant melanoma and demonstrated an 89% partial response lasting about a year and several mixed responses, with no dose-limiting side effects. They suggest that anti-TGF- β antibodies could be combined with vaccines. Berzofsky and colleagues have conducted a phase I trial of an epitope-enhanced prostate cancer antigen called TARP in patients with D₀ prostate cancer, finding that

the vaccine significantly reduced PSA rate of increase in 74% of patients at 48 weeks. A phase II study is being planned.

Summary

The 21st annual CRI cancer immunotherapy symposium, entitled "Dynamics of Host-Tumor Interaction," was held in New York City from September 30 through October 2, 2013, and featured 27 presentations on tumor immunology by leading researchers, many of them supported by CRI. Topics included the role of inflammation in promoting tumorigenesis and tumor progression, interactions between the cancer stroma and immune cells in the tumor microenvironment and during metastasis, the role of microbes in cancer, and the role of immune regulators in modulating antitumor responses. Michael Karin, recipient of the 2013 Coley Award, gave the inaugural William B. Coley lecture as the keynote address.

– *Matthew Tontonoz*
Cancer Research Institute, New York, NY

– *Connie E. Gee*
AACR, Philadelphia, PA

Published online February 4, 2014.

References

- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
- Keenan BP, Jaffee EM, Armstrong TD. Tumor immunology: multidisciplinary science driving basic and clinical advances. *Cancer Immunol Res* 2013;1:16–23.
- The 2013 William B. Coley Award for Distinguished Research in Basic and Tumor Immunology. *Cancer Immunol Res* 2013;1:362–3.
- Mandi JN, Liou R, Klauschen F, Vriskoop N, Monteiro JP, Yates AJ, et al. Quantification of lymph node transit times reveals differences in antigen surveillance strategies of naive CD4+ and CD8+ T cells. *Proc Natl Acad Sci U S A* 2012;109:18036–41.
- Barkauskas DS, Evans TA, Myers J, Petrosiute A, Silver J, Huang AY. Extravascular CX3CR1+ cells extend intravascular dendritic processes into intact central nervous system vessel lumen. *Microsc Microanal* 2013;19:778–90.
- Marangoni F, Murooka TT, Manzo T, Kim EY, Carrizosa E, Elpek NM, et al. The transcription factor NFAT exhibits signal memory during serial T cell interactions with antigen-presenting cells. *Immunity* 2013;38:237–49.
- Chou J, Lin JH, Brenot A, Kim JW, Provot S, Werb Z. GATA3 suppresses metastasis and modulates the tumour microenvironment by regulating microRNA-29b expression. *Nat Cell Biol* 2013;15:201–13.
- Seo AN, Lee HJ, Kim EJ, Kim HJ, Jang MH, Lee HE, et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. *Br J Cancer* 2013;109:2705–13.
- Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol* 2013;31:860–7.
- Zal T, Chodaczek T. Intravital imaging of anti-tumor immune response and the tumor microenvironment. *Semin Immunopathol* 2010;32:305–17.
- Blatner NR, Gounari F, Khazaie K. The two faces of regulatory T cells in cancer. *Oncol Immunology* 2013;2:e23852.
- MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* 2013;31:259–83.
- Sukumar M, Liu J, Subramanian M, Crompton JG, Yu Z, Roychowdhuri R, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* 2013;123:4479–88.
- Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 2013;153:1239–51.
- Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *Am J Med Sci* 1893;105:487.
- Hess DJ. Can bacteria cause cancer? Alternative medicine confronts big science. New York, NY: New York University Press, 1997.
- Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013;14:207–15.
- Kostic AD, Chun E, Meyerson M, Garrett WS. Microbes and inflammation in colorectal cancer. *Cancer Immunol Res* 2013;1:150–7.
- Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 2013;342:967–70.
- Baker K, Qiao SW, Kuo TT, Aveson VG, Platzer B, Andersen JT, et al. Neonatal Fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8-CD11b+ dendritic cells. *Proc Natl Acad Sci U S A* 2011;108:9927–32.
- Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8+ T cells. *Nat Rev Immunol* 2011;11:645–57.
- O'Sullivan T, Saddawi-Konefka R, Vermi W, Koebel CM, Arthur C, White JM, et al. Cancer immunoediting by the innate immune system in the absence of adaptive immunity. *J Exp Med* 2012;209:1869–82.

23. Miyoshi H, Ajima R, Luo CT, Yamaguchi TP, Stappenbeck TS. Wnt5a potentiates TGF- β signaling to promote colonic crypt regeneration after tissue injury. *Science* 2012;338:108–13.
24. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; 315:1650–9.
25. Jaiswal S, Chao MP, Majeti R, Weissman IL. Macrophages as mediators of tumor immunosurveillance. *Trends Immunol* 2010;31:212–9.
26. Tseng D, Volkmer J-P, Willingham SB, Contreras-Trujillo H, Fathman JW, Fernhoff NB, et al. Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective anti-tumor T-cell response. *Proc Natl Acad Sci* 2013;110:11103–8.
27. Kärre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. *Nature* 1986;319:675–8.
28. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443–54.
29. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 2002;298: 1395–401.
30. Malchow S, Leventhal DS, Nishi S, Fischer BI, Shen L, Paner GP, et al. Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* 2013;339:1219–24.
31. Berrien-Elliott MM, Jackson SR, Meyer JM, Rouskey CJ, Nguyen TM, Yagita H, et al. Durable adoptive immunotherapy for leukemia produced by manipulation of multiple regulatory pathways of CD8+ T-cell tolerance. *Cancer Res* 2013;73:605–16.
32. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013;210:1695–1710.
33. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013;369:122–33.