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Metabolic Reprogramming of the Immune Response in the Tumor Microenvironment

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A Division of Cancer Biology, NCI-sponsored workshop Metabolic Reprogramming of the Immune Response in the Tumor Microenvironment was held October 2nd, 2012 in Bethesda, MD. The purpose of the workshop was to bring together cancer cell biologists and immunologists to explore the mechanistic relationships between the metabolic pathways used by cancer cells and antitumor immune cells and how this information could be used to improve cancer immunotherapy. At the conclusion of the workshop a general discussion focused on defining the major challenges and opportunities concerning the impact of metabolism on antitumor immunity and cancer immunotherapy as well as what tools, technologies, resources or community efforts are required to accelerate research in this area. Overall, future studies need to consider how cancer cell metabolic pathways differ from activated lymphocytes in order to define a therapeutic window for cancer therapy. Further, studies aimed at reprogramming the metabolic qualities of T cells with the goal of improving immunotherapy were considered a promising avenue.

Introduction

Our understanding of how immune responses are generated, propagated and stored as memory is undergoing a radical transformation. Recent groundbreaking studies have demonstrated that immune activation, acquisition of effector functions and generation of immune memory are coupled with profound changes in cellular metabolism. In particular, the transition from quiescence to activation is coupled with a significant and prolonged upregulation of aerobic glycolysis (Warburg metabolism) relative to mitochondrial oxidative phosphorylation (OXPHOS). This metabolic reprogramming to aerobic glycolysis is necessary for both innate and adaptive immune functions. Further, the development of long-lived central memory T cells requires a metabolic switch back to mitochondrial OXPHOS or fatty acid oxidation energy production. Since robust antitumor memory T-cell development is correlated with cancer regression, these new mechanistic details pertaining to the metabolic requirements of T-cell activation and memory T-cell development hold

promise to dramatically alter how immune responses are evoked by manipulation of the metabolic machinery. The principal goal of this workshop was to catalyze further progress in understanding the role of cellular metabolism in achieving effective antitumor immunity.

Opening talks addressed the variety of metabolic platforms observed in cancers, the nutrient uptake and metabolic pathways that support cancer cell growth and technologies to visualize and target cancer cell vulnerabilities. Matthew Vander Heiden (MIT) evaluated the causes and consequences of the metabolic switch from mitochondrial respiration to aerobic glycolysis in proliferating cells including cancer cells and activated immune cells. A prevailing thought is that this metabolic switch has less to do with ATP production than with how proliferating cells acquire the biosynthetic precursors to generate the building blocks (proteins, lipids and nucleotides) to fuel cell expansion. Cancer cells are capable of utilizing a variety of nutrient substrates. Both substrate selection and the microenvironment contribute to determining a cell's metabolic phenotype. For example, although cancer cells synthesize lipids from multiple carbon sources including glucose and glutamine, glutamine is the principal source under hypoxic conditions. Thus, one characteristic of rapidly proliferating cells is metabolic plasticity. Given that tumors are characterized as having a poor blood supply, such metabolic plasticity would allow cancer cells to utilize alternative nutrient sources to support their biosynthetic needs. Glucose is a major substrate for most cells and is regulated by pyruvate kinase (PK). Most cells express one of two major PK isoforms: a highly active PK-M1 tetramer or less active PK-M2 monomer. Expression of low activity PK-M2 generates glycolytic intermediates, such as lactate, and is permissive for tumor growth. In contrast, PK-M1 neither supports proliferation nor generates much lactate, and overexpression of PK-M1 in tumor cell lines suppresses tumor growth *in vivo* in mice.¹

Douglas Green (St. Jude Children's Research Institute) continued the theme of how cancer cells support the energy and biosynthetic demands of cellular proliferation focusing on macroautophagy. Both phagocytosis and autophagy are ancient biological processes that likely co-evolved to cope with various metabolic or immune-mediated environmental stressors. Therefore, it shouldn't be surprising that some components of the phagocytic and autophagic pathways are shared. A novel mechanism of autophagy-associated phagocytosis was presented that enabled efficient uptake and clearance of dead cells. Whether

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autophagy-associated phagocytosis plays a role in reprocessing protein aggregates, cell fragments or corpses to provide energy and biosynthetic precursors for proliferating cells is not clear. However, processing these components down to simple metabolites would provide all the building blocks required by growing cells.

Brian Altman (University of Pennsylvania) focused on the role of the transcription factor c-Myc in regulating cell growth and metabolism. Myc stands at the crossroads of nutrient accumulation and generation of biomass through its regulation of genes involved in cell growth and differentiation. Recent metabolic profiling has suggested flexibility in how cancer cells utilize different metabolic pathways. Under normoxic conditions, Burkitt lymphoma cells expressing high c-Myc levels, primarily utilize glutamine. However, under hypoxia, glucose metabolism goes up, suggesting that cancer cells have some flexibility in how they can process nutrients based upon c-Myc levels and the availability of oxygen in the microenvironment.² Taken together, these studies suggest a framework to consider how metabolism is regulated to support various cell functions and will inform future studies targeting metabolism for therapy.

Ralph J. DeBerardinis (UT-Southwestern Medical Center) described approaches utilizing mass spectrometry and NMR spectroscopy to track metabolic pathway choice in living tissues. Metabolic flux analysis of human lung cancer cell lines *in vitro* identified at least three different metabolic platforms that enable high growth rates in culture: glutamine-dependent anaplerosis, glutamine-dependent reductive carboxylation and glucose-dependent anaplerosis. These data reinforce the concept that there are numerous metabolic platforms that can support cancer cell growth in culture and imply that determining which of these pathways are active *in vivo* will greatly improve the chances for successful metabolic cancer therapies.

Caius Radu (UCLA) highlighted the *de novo* and nucleoside salvage pathways used by proliferating cells, including cancer cells and lymphocytes, to generate the deoxyribonucleotide triphosphate (dNTP) pools necessary to support DNA replication and repair. Both pathways can be imaged using positron emission tomography (PET) probes for non-invasive imaging of nucleotide metabolism at a whole body level in mice and humans.³ While both *de novo* and nucleoside salvage pathways generate dNTPs, their precise contributions to the expansion of dNTP pools occurring during T-cell development and activation are yet to be defined. It is also unknown whether there are differences between proliferating T lymphocytes and cancer cells. Elucidating these aspects would contribute to a broader understanding of how dividing cells regulate the metabolic flux through the *de novo* and nucleoside salvage pathways and how these two biosynthetic pathways can be modulated therapeutically to induce synthetic lethality in hematological malignancies and other cancers.

Next, the relationship between metabolism and lymphocyte activation, expansion and effector function was explored. The role of the central effector molecule, mTOR, in regulating the glycolytic shift to support lymphocyte expansion and ability to augment antitumor immunity were addressed. Jeffrey Rathmell (Duke University) focused on the role of glucose uptake and

metabolism in regulating T-cell proliferation and differentiation. The glucose transporter family is comprised of 14 member, with Glut1 serving as the principal glucose transporter. T cells primarily express Glut1 and Glut3, although Glut6 and Glut8 are also expressed. However, upon activation T cells switch to predominantly Glut1, commensurate with increased glucose uptake. Microarray analyses have also revealed that each T-cell subset is metabolically distinct. Whereas activated Th1, Th2 and Th17 subsets are highly glycolytic, Treg utilize a more oxidative metabolism with lipids serving as a principal fuel.⁴ These metabolic distinctions may allow a new understanding and approaches to manipulate immunity *in vitro* and *in vivo*.

Erika L. Pearce (Washington University School of Medicine) presented studies supporting a critical need to reassess the basic metabolic requirements for T-cell proliferation, survival and effector function. Numerous studies have demonstrated that activated T cells dramatically increase glucose uptake and lactate production, indicating that aerobic glycolysis is engaged. However, oxygen consumption is also increased under activating conditions. These findings suggest that T cells engage both OXPHOS and Warburg metabolism during activation.⁵ Further, although activated cells switch to Warburg metabolism, presumably to support cell expansion, dendritic cells and macrophages also switch to Warburg metabolism upon activation even though they do not proliferate. This observation suggests that the conventional interpretation that Warburg metabolism is required to support the bioenergetic demands of proliferation is not fully correct. Thus, the metabolic requirements for T-cell activation, expansion and effector function need to be reevaluated.

Jonathan Powell (Johns Hopkins School of Medicine) discussed the role of the serine/threonine protein kinase mTOR in regulating T-cell development. mTOR regulates nutrient and hormone-sensitive signaling pathways and has been implicated in integrating signals from the microenvironment to instruct T-cell differentiation and effector development programs. Previous studies using genetically engineered mouse models have shown that mTORC1 and mTORC2 selectively regulate CD4⁺ T-cell differentiation. The development of Th1 and Th17 cells is dependent on mTORC1. In contrast, mTORC2 is necessary for the differentiation of Th2 cells. When either mTORC1 and mTORC2 complexes are genetically eliminated or mTOR is pharmacologically blocked, the default differentiation pathway is toward Treg cells. The downstream targets of mTORC1 and mTORC2 that regulate CD4⁺ T-cell differentiation have not been identified, but these data suggest that metabolic manipulation could skew the immune response.⁶ Thus, an emerging concept is that metabolic considerations play a critical role in determining the developmental outcome subsequent to lymphocyte activation.

Edward J. Pearce (Washington University School of Medicine) discussed the role of mTOR in dendritic cell (DC) survival and activation. Toll-like receptor (TLR) agonists activate DCs and upregulate the expression of co-stimulatory receptors, antigen processing and presenting machinery, cytokines and chemokines. This fundamental process is also accompanied by increased glucose uptake and an increase in aerobic glycolysis. The switch to glycolysis in activated DCs is also accompanied by a marked

increase in the expression of the inducible form of nitrate oxide synthase (iNOS) and generation of nitric oxide (NO). The NO creates an anaerobic environment that cripples mitochondria and inhibits OXPHOS. Because OXPHOS is inhibited in activated DCs, these cells are highly glucose dependent and short-lived. Inhibiting the glycolytic switch, by adding rapamycin, augments the ability of DCs to activate T cells. In the presence of rapamycin, DCs rely on OXPHOS to serve their energy demands and do not upregulate iNOS nor generate NO. Thus, in the presence of rapamycin, NO production is decreased and mitochondrial function is maintained. Consequently, TLR-activated monocyte-derived DCs treated with mTOR inhibitors are long-lived and capable of interchangeably using different substrates to support their metabolic needs.⁷ These findings define mTOR as a molecular target for augmenting DC survival and activation.

Rafi Ahmed (Emory Vaccine Center) examined the role of mTOR in regulating B-cell responses. Rapamycin treatment led to a greater than 10-fold inhibition of the primary antibody response to acute infection with lymphocytic choriomeningitis virus (LCMV). Germinal center formation was decreased and both plasma and memory B cells were reduced. T follicular helper (Tfh) cells provide primary help to B cells and are required for germinal center formation. Paradoxically, Tfh cell-specific knockdown of mTOR promoted Tfh cell development. However, the germinal center response to LCMV challenge was rescued using rapamycin-insensitive B cells suggesting that during a primary response, rapamycin acts to inhibit B-cell proliferation which impairs the differentiation of naïve CD4 T cells into Tfh cells and reduces germinal center formation. However, giving rapamycin 90 d after LCMV challenge had no effect indicating that recall responses are not affected by rapamycin. Thus, mTOR plays an important role in regulating the proliferation, longevity and development of B-cell immune responses.

Doreen Cantrell (University of Dundee) detailed how mTOR serves as a central regulator of cytotoxic T lymphocyte (CTL) function. Whole cell proteomic analysis of CTL using mass spectrometry identified 5,000–6,000 proteins in T cells whose expression is changed in the presence of rapamycin. Rapamycin-treated T cells downregulate expression of glucose transporters Glut1 and Glut3 and glycolytic enzymes whereas proteins involved in the TCA cycle and mitochondrial respiration were increased. Expression of CCR7 and CD62L which regulate the migration of naïve T cells to lymphoid tissue is restored by rapamycin in effector CTL, permitting them to home back to secondary lymphoid tissues where they are not normally found. Thus, rapamycin treatment reprograms T-cell metabolism at multiple levels. It is likely that the mTOR pathway controls T-cell metabolism and trafficking via regulation of HIF1 α . HIF1 α plays a critical role in regulating glucose metabolism, expression of cytolytic effector molecules and chemokine and adhesion receptors that control migration.⁸

Nicholas Restifo (NCI) discussed approaches whereby reprogramming of the metabolic qualities of T cells would lead to improved antitumor immunotherapies. Adoptive T-cell immunotherapy has the potential for complete eradication of tumor burden. Current adoptive T-cell immunotherapy uses either

autologous tumor infiltrating lymphocytes (TILs) or genetically engineered T cells that are expanded ex vivo and adoptively transferred back to the patient. There has been intense interest in defining the specific qualities of transferred T cells that produce a complete response vs. those that generate no response or a partial response. The most important quality is that T cells persist in vivo after adoptive transfer. Although effector T cells lose the potential for self-renewal and inevitably senesce, naïve and memory T cells possess the capacity for self-renewal. Thus, although short-lived T effector cells may be efficient cytotoxic killers, the long-term therapeutic efficacy of adoptively transferred cells resides in the naïve and memory pool. Consistent with this observation, inhibiting the glycolytic switch with 2-deoxy-D-glucose (2DG) prevented senescence and led to greater survival advantage of adoptively transferred T cells in vivo.⁹ These findings inform a novel approach whereby if T cell metabolism could be reprogrammed to be less glycolytic during expansion the capacity for self-renewal would be preserved in vivo. Future studies will focus on reprogramming the metabolic qualities of T cells in adoptive cellular immunotherapy, with the goal of generating long-lived antitumor T cells.

At the conclusion of the workshop, a general discussion focused on defining the major challenges and opportunities concerning the impact of metabolism on antitumor immunity and cancer immunotherapy and what tools, technologies and community resources are required to accelerate research in this area. Overall, future studies need to consider how cancer cell metabolic pathways differ from activated lymphocytes in order to define the therapeutic window to improve immunotherapy.

Major Challenges and Opportunities

- There is a critical need to assess the crosstalk between the different metabolic platforms that may be operating in any given cell and to integrate those assessments to generate a more complete metabolic profile at the single cell level.
 - The relative contributions of biosynthetic molecules taken up from the environment vs. those generated de novo and how these pathways affect cancer progression need to be determined.
 - The relationship between metabolism or different metabolic platforms and self-renewal capacity need to be determined.
 - A better understanding of how the metabolic environment (or nutrient repertoire) in normal tissues, immune tissues and in the setting of pathology (in tumors) affects immune cell development and/or effector function is needed.
 - Studies to elucidate how specific metabolites affect various immune states such as activation, anergy, development of long-lived memory cells vs. short-lived effector cells and homing to their proper niche are needed.
 - The consequences of metabolic interventions on immune cell growth and effector functions need to be fully understood.

Tools, Technologies and Community Resources

- New instrumentation and novel approaches are needed to parse the metabolic heterogeneity of tissues in situ and establish the

metabolic changes that are occurring at the single cell level in vivo.

- Improved methods and approaches need to be developed to assess how the metabolic characteristics of the tumor microenvironment affect the antitumor immune response.

- Improved methods and approaches are required to address whether reported symbioses or competition for metabolite pools actually occur in the tumor microenvironment in vivo.

- There is a need for computational models to better understand which metabolic pathways are hard-wired and when perturbed lead to cell death and which are flexible and can be experimentally manipulated.

- There is a critical need to develop small molecule inhibitors of glycolysis, FAO and other metabolic pathways for therapeutic intervention.

- Lastly, a comprehensive analysis of the metabolic heterogeneity (metabolic flux/metabolomics) of the different immune subsets and a full description of the functional consequences of differential utilization of metabolic pathways in supporting immune cell differentiation is needed.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest have been disclosed.

Attendees

Rafi Ahmed (Emory University School of Medicine, Atlanta, GA), Brian Altman (University of Pennsylvania, Philadelphia, PA), Doreen Cantrell (University of Dundee, Dundee, Scotland, UK), Ralph J. DeBerardinis (UT-Southwestern Medical Center, Dallas, TX), Douglas Green (St. Jude Children's Research Institute, Memphis, TN), Kevin Howcroft (National Cancer Institute, NIH, Bethesda, MD), Susan A. McCarthy, (National Cancer Institute, NIH, Bethesda, MD), R. Allan Mufson, (National Cancer Institute, NIH, Bethesda, MD), Edward J. Pearce (Washington University School of Medicine, St. Louis, MO), Erika L. Pearce (Washington University School of Medicine, St. Louis, MO), Jonathan Powell (Johns Hopkins School of Medicine, Baltimore, MD), Caius Radu (UCLA, Los Angeles, CA), Jeffrey Rathmell (Duke University, Durham, NC), Nicholas P. Restifo (National Cancer Institute, NIH, Bethesda), Dinah Singer (National Cancer Institute, NIH, Bethesda) and Matthew Vander Heiden (MIT, Cambridge, MA).