Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

Perspective

Cancer Research

The Emerging "Hallmarks" of Metabolic Reprogramming and Immune Evasion: Distinct or Linked?

Irina Kareva and Philip Hahnfeldt

Abstract

The role of the immune system in tumor elimination has been shown to be increasingly ambiguous, as many tumors not only escape recognition by the adaptive immune response but also even prime the immune cells to promote tumor growth. This effect is achieved through a number of mechanisms, which include both direct interference with the cells of the adaptive immune response and indirect immunosuppression achieved through modification of the tumor microenvironment. We propose that through upregulation of glycolysis and the consequent lowering of pH in the tumor microenvironment, tumors can take advantage of a pH control system, already exploited by specific immune cell subpopulations, to gain control of the immune system and suppress both cytotoxic and antigen-presenting cells. This is accomplished through the direct competition of tumor cells with actively proliferating glycolytic immune cells for glucose and indirectly through the creation by the tumor of a microenvironment that interferes with maturation and activation of antigen-presenting cells and naïve cytotoxic T cells. Immunosuppressive properties of an acidic microenvironment in the vicinity of the tumor can thus provide additional benefits for upregulation of glycolysis by tumor cells, suggesting that the two emerging "hallmarks of cancer," altered glucose metabolism and immune suppression, are in fact fundamentally linked. *Cancer Res; 73(9); 2737–42. ©2013 AACR.*

Introduction

Tumors have evolved numerous ways to escape recognition by the adaptive immune system. These involve suppressing the adaptive immune response at the level of antigen presentation through promotion of expansion of immunosuppressive myeloid-derived suppressor cells (MDSC) while preventing their maturation to functional antigen-presenting cells, including dendritic cells (DC; ref. 1), and by promoting the expansion of tumor-associated macrophages through upregulated expression of interleukin (IL)-4, IL-10, IL-13, and glucocorticoid hormones (2, 3). Interestingly, low pH has been seen to influence the balance of function among immune subpopulations, including expansion of immunosuppressive MDSCs through stabilization of hypoxia-inducible factor (HIF)-1α, as well as direct the priming of naïve T cells toward the tumor-promoting T-helper (T_H)2 phenotype and away from the antitumor T_H1 phenotype by way of denaturation of acid-labile IFN-γ (4, 5). Here, we propose this same ability of low pH to dictate the actions of immune subpopulations may be exploited by tumors to circumvent the antitumor immune response through the upregulation of glycolysis and avoidance of oxidative phosphorylation by tumor cells. It is noted that such mechanisms may be undermining the efficacy of existing immunotherapies.

Authors' Affiliation: Center of Cancer Systems Biology, St. Elizabeth's Medical Center, Tufts University School of Medicine, Boston, Massachusetts

Corresponding Author: Philip Hahnfeldt, Center of Cancer Systems Biology, St. Elizabeth's Medical Center, Tufts University School of Medicine, 736 Cambridge St., CBR-115, Boston, MA 02135. Phone: 617-789-2998; Fax: 617-562-7142; E-mail: Philip.Hahnfeldt@tufts.edu

doi: 10.1158/0008-5472.CAN-12-3696

Overview of Normal Immune Response

As hematopoietic stem cells leave the bone marrow, they differentiate either into lymphoid progenitors that migrate to the thymus, where they further differentiate into T cells, or into myeloid progenitors that differentiate into monocytes and migrate to tissues, where they further differentiate into immature myeloid cells, such as DCs and macrophages. Once immature DCs encounter an antigen, they internalize it, display its fragments on the cell surface, and migrate to lymph nodes, where they can activate naïve T cells. DCs mature, that is, they lose their ability to engulf pathogens and develop an increased ability to communicate with T cells, during migration. Cytotoxic T cells activated by DCs undergo rapid clonal expansion and then migrate throughout the body in search of cells exhibiting the corresponding antigens. T cells conduct their cytotoxic function either via secretion of perforin or similar substances that damage the cell membrane and cause the cell to swell and lyse or through inducing apoptosis, which causes the target cell to shrink and die (6, 7).

Hematopoietic stem cells that do not migrate to the thymus but remain to mature in the bone marrow yield antigen-specific precursors of B cells, which then migrate to secondary lymphoid organs, such as lymph nodes and spleen. Upon recognition of antigens by B-cell receptors, B lymphocytes become activated and undergo rapid clonal expansion, gaining an augmented ability to recognize foreign antigens (7). While

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

B lymphocytes are primarily associated with antigen recognition and presentation, they have also been shown to promote a chronic inflammatory state associated with premalignant progression (6).

MDSCs are a heterogeneous population of immunosuppressive cells of myeloid lineage (1), whose concentration has been shown to be increased up to 10-fold in patients with cancer (8, 9). As will be seen, MSDCs play an important role in the connection between tumor metabolic reprogramming and immune evasion.

Overview of Glucose Metabolism in Normal and Cancer Tissues

All the cells in the human body, whether benign or malignant, depend on metabolizing glucose both for proliferation and physiologic maintenance. Once ingested via nutrient transporters, glucose is broken down into pyruvate and two molecules of ATP. In the presence of oxygen, pyruvate enters the mitochondrion of the cell, where it is oxidized via the Krebs cycle, yielding up to 30 additional molecules of ATP. Otherwise, pyruvate is converted to lactate by the enzyme lactate dehydrogenase (LDH) and is then released from the cell. With respect to the exploitation by the tumor or immune cells of "glycolysis" or the "glycolytic phenotype," we will, in deference to literary precedent, be referring henceforth to a process that converts glucose to lactate, rather than pyruvate. This is done with the understanding that normal glycolysis is not a pathway that deviates from oxidative phosphorylation but links to it when the latter is operative.

While energetically inefficient, glycolysis importantly can provide cells with glycolytic intermediates necessary for biosynthesis of DNA and cell structural material. This is done via the pentose phosphate pathway and involves generating NADPH, which is then used for fatty acid synthesis, and ribose-5-phosphate, which is used for synthesis of nucleotides (10). That said, a special property of cancer cells is their frequent persistence in using glycolysis even in the areas of ample oxygen supply capable of supporting oxidative phosphorylation, a phenomenon known as the Warburg effect. As will be discussed, this could confer on cancer cells an ability to synthesize various macromolecules needed to sustain high proliferation rates and otherwise compete with the immune population on its own terms, as actively proliferating cells of the immune system also bypass oxidative phosphorylation during glucose metabolism even in areas of ample oxygen supply.

Activated Immune Cells Engage in Glycolytic Metabolism to Facilitate Expansion

To accomplish their cytotoxic function, activated immune cells need to undergo clonal expansion, a very energy-demanding process (11). To meet this demand, it has been observed that activated T cells can hyperinduce glycolysis quickly, increasing uptake as much as 20- to 40-fold as cells prepare to divide (12) to reach levels of consumption where an estimated 85% of glucose supply is excreted by T cells as lactate (13). To permit production of the necessary complex macromolecules for proliferation, transition from the resting to the activated state for T cells causes a switch from catabolic to anabolic metabolism. This involves the active suppression of oxidation of amino acids and lipids, diverting them to be used, along with glucose metabolites, as building materials for cell expansion (11). In contrast, failure to increase glucose metabolism during lymphocyte activation prevents cell growth and prevents production of cytokines such as IFN-γ, which are required for effector function (14). Notably, activated T cells switch to glycolysis under both hypoxic and normoxic conditions, suggesting that, just as in cancer cells that undergo matching metabolic shifts (15), it is the speed of nutrient uptake and the increased ability for fast proliferation that drive the shift in metabolic strategy for immune cells (11).

Resting T cells, on the contrary, derive most of their ATP from oxidative phosphorylation. Although quiescence is not as energy demanding as proliferation, it is still an actively maintained state: ATP generated via oxidative phosphorylation is very likely used to actively suppress, by upregulated protein degradation, the expression of cell-cycle proteins (11). However, without cytokine-dependent signals from their microenvironment, such as IL-2 or IL-7, even resting cells cannot take up nutrients, and thus die by starvation as they internalize Glut1 and other nutrient transporters (14). From an evolutionary point of view, this could arguably be serving as a protective mechanism for the host to ensure that immune cells do not compete for nutrients with somatic cells unless they are conducting their function. Notably, cancer cells do not require any additional signals from their microenvironment to enable them to take up nutrients, providing them with a possible additional competitive advantage in the event of low tumor immunogenicity.

DCs have also been shown to switch to glycolysis in an activated state (16). However, in the tumor microenvironment, their activation is often suppressed, yielding an expansion of the population of immature myeloid cells, also known as MDSCs.

Tumor–Immune Interaction Is Modulated by Microenvironmental Acidification Due to Glycolysis Dependence

Lowered pH due to glycolysis dependence can prevent HIF-1α denaturation under normoxia

A possible link between immune modulation and glycolysis dependence of the tumor–immune system lies in the body's response to hypoxia, which is often observed in tumor microenvironments as a result of upregulated cell growth and insufficient vascularization. Exposure to hypoxic conditions causes the cells to upregulate production of HIF-1, which is a heterodimer that consists of an oxygen-sensitive HIF-1α subunit and a constitutively expressed HIF-1β subunit (17, 18). In the absence of oxygen, HIF-1α binds to hypoxia response elements (HRE), activating the expression of numerous hypoxia response genes, including VEGF (19) and other angiogenesis-promoting factors that stimulate blood flow and bring oxygen to hypoxic areas. In the presence of oxygen, HIF-1α normally binds to the tumor suppressor von Hippel-Lindau (VHL) protein, which causes the ubiquitylation and subsequent degradation of HIF-1α by proteasomes (18).

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

The environmental effect of glycolysis dependence may work predominantly through lowered pH. Mekhail and colleagues (20) have shown that both hypoxia and normoxic acidosis can neutralize the function of VHL protein, which suggests that an acidic microenvironment, such as can be observed in many tumors that upregulate glycolysis and increase their production of lactic acid, can "simulate" the effects of hypoxia even in the presence of an adequate oxygen supply. This effect has been observed in the 5.8 to 6.6 pH range, whereas many hypoxic tumors have pH ranges from 6.4 to 7.0 (21). Consequently, in low pH, created as a byproduct of upregulated glycolysis, HIF-1α may not be degraded even in the presence of an adequate oxygen supply, resulting in increased production of VEGF and increased angiogenesis. This, in turn, could stimulate expansion of MDSCs (1). Expansion of the MDSC population both within the tumor microenvironment and at its periphery results in upregulated expression of immunosuppressive factors such as arginase (arg1), as well as in an increased production of nitric oxide and reactive oxygen species (ROS). Increased activity of arg1 in MDSCs leads to enhanced L-arginine catabolism; the consequent shortage of L-arginine can inhibit $CD8^+$ T-cell function (22). Nitric oxide can suppress T-cell function through inhibition of MHC-II expression and T-cell proliferation and apoptosis (23, 24). Tumor-derived factors, such as TGF-β, induce production of ROS by MDSCs; inhibition of ROS production has been shown to nullify the suppressive effect of these cells (25). Interestingly, peripheral MDSCs migrating to the tumor site may themselves differentiate into tumor-associated macrophages (TAM), which produce cytokines that can further suppress T-cell responses in a nonspecific manner (1). MDSCs can also suppress cytotoxic lymphocytes by not differentiating into functional dendritic cells and thus not fulfilling their antigen-presenting function. Moreover, Corzo and colleagues (26) showed that in the tumor microenvironment, upregulation of HIF-1α can also promote MDSC differentiation toward TAMs, which in turn suppress both specific and nonspecific T-cell functions. Doedens and coworkers (27) further showed that loss of HIF-1α in myeloid cells directly counters hypoxia-induced suppression of T-cell activation and leads to slowed tumor progression. Interestingly, permanent dysfunction of VHL protein, which results from loss-of-function mutations, can cause VHL disease, characterized by sporadic hemangioblastomas found in kidney, cerebellum, retina and spinal cord, and renal clear cell carcinomas (28).

In summary, the acidic microenvironment created in the tumor vicinity by glycolytic activity can cause upregulated expression of HIF-1α and VEGF, both of which can contribute not only to increased angiogenesis but also to decreased antigen presentation and the expansion of the population of immunosuppressive MDSCs.

Lowered pH due to glycolytic activity can promote tumor growth through inhibition of IFN-γ and TNF

Another way by which tumors can suppress immune function is through interfering with maturation of regulatory T cells. One possible mechanism can be traced to IFN-γ, which is a cytokine produced by natural killer (NK) cells of the innate immune system and by cytotoxic T lymphocytes (CTL; ref. 29). It is one of the primary mediators of both innate and adaptive immunity, playing a key role in macrophage activation, promoting adhesion and binding required for leukocyte progression, and modulating the differentiation both of T_H and regulatory T (Treg) cells (29, 30).

T cells consist of $CD8^+$ cytotoxic cells and $CD4^+$ T_H cells, the latter of which are further subdivided into T_H1 cells that primarily express IFN-γ and TNF-α, and T_H2 cells expressing IL-4, -5, and -13 (5). T_H1 cells maximize the killing efficiency of macrophages and proliferation of $CD8^+$ T cells and inhibit production of IL-4. T_H2 cells can stimulate B cells, which have been associated with tumor-promoting inflammatory responses (6), although T_H2 cell differentiation itself is inhibited by IFN-γ (30). Indeed, mice with inhibited IFN-γ signaling display increased T_H2 polarization and are consequently more susceptible to tumor development (31). *In vitro*, IL-4 expression increases expression by TAMs of EGF, which improves entry of cancer cells into the circulation (5). IFN-γ promotes activation of M1, or "classically activated" macrophages, which are involved in combating acute-phase parasitic infections (2–4, 32) and antitumor immunity (4). Meanwhile, M2, or "alternatively activated" macrophages, are stimulated by IL-4, -13, and -10 (3, 4, 31), are associated with tissue repair (2), and encourage tumor progression (4).

As it so happens, IFN-γ is acid labile and can be denatured in low pH (33), which will likely cause T-cell maturation to be diverted away from the antitumor T_H1 phenotype toward the protumor T_H2 phenotype, as well as prevent the activation of tumoricidal M1 macrophages. Further support for a link between immune competency and expression of IFN-γ and TNF-α may be drawn from a study by Barth and colleagues (34), who showed using mice irradiated to abolish cytotoxic lymphocytes, that the adoptive transfer of noncytolytic $CD8^+$ tumor-infiltrating lymphocyte (TIL) cultures into these hosts can bring about regressions of established tumors. Interestingly, they found (i) that the effectiveness of the cultures related more to their production of IFN-γ and TNF-α than to their direct cytotoxicities and (ii) that antibodies to IFN-γ and TNF-α inhibited their antitumor potencies independently of their cytotoxicities. Suggested was a novel delayed-type hypersensitivity (DTH) reaction mechanism for IFN-γ- and TNF-α–dependent $CD8^+$ antitumor action involving stimulated resident macrophages as the final effectors of the DTH. In a second study coupling acidity with macrophage action (35), it was found that TNF-α secretion by activated alveolar macrophages was reduced for lower pH values. Taken together, these findings may be suggesting that sufficient acidification of the tumor microenvironment, with accompanying inactivation of IFN-γ and/or suppression of TNF, may encourage tumor progression by limiting the maturation and function of T_H1 lymphocytes and M1 macrophages, while encouraging the expansion of tumor-promoting T_H2 lymphocytes.

Humoral and Nonadaptive Immune Effects

Beyond the generally immunosuppressive action usually attributed to low pH, evidence suggests it may also be a differential regulator of immune function, promoting certain

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

humoral and cellular immune activities as well (36). Indeed, Lopez and colleagues (37) showed that acidic pH increases the activity of human immunoglobulin G (IgG) binding to human neutrophils, monocytes, and NK cells. Furthermore, as shown by a number of studies (38–43), increased activity of the complement system is also observed at lower pH. Trevani and colleagues (44) showed delayed apoptosis of neutrophils in low pH, as well as increased binding of neutrophils to endothelial cells during inflammation via upregulated expression of surface molecules, such as CD18. Some years later, Tong and colleagues (45) showed that exposure of cells to pH 6.5 can in fact promote maturation of DCs through significantly upregulated expression of CD11c and MHC class II, as well as costimulatory molecules CD86 and CD80, an effect that is not observed at pH 7.3. Other groups (46, 47) have also reported increased activation and improved efficacy of DCs cultured in acidic conditions, accompanied by increased production of T_H1-associated cytokines, such as IL-12, as well as MHC class I molecules. One may conclude the role of extracellular acidosis is not clearly immunosuppressive, as is commonly believed, but that acidosis can have both promoting and suppressive effects on different classes of immune cells.

Aerobic Glycolysis by Tumor Cells Can Promote Immune Evasion

A preponderance of evidence therefore suggests that tumor development in the immune context can be modulated by environmental acidification made possible by tumor adoption of the same glycolytic dependency as seen in the immune system. In turn, as the immune system can both be promoted and suppressed by the microenvironmental consequences of glycolysis, tumor cooption of these same responses through adoption of a glycolytic program provides the makings of an elaborate competitive dynamic. All of these considerations are summarized in Figure 1. Interestingly, the observed abilities of cancer cells to evade the immune system and reprogram their energy metabolism have recently been identified as distinct "emerging hallmarks of cancer" (48). Given the shared strategic dependence of both cancer and immune systems on glycolysis, however, the two hallmarks would appear fundamentally linked. Both immune and cancer cells can hyperinduce glycolysis for glucose metabolism when they switch to a highly proliferative state, satisfying a need shared by both systems for large amounts of readily available energy and building materials (10). However, while unactivated immune cells are largely dependent on oxidative phosphorylation (11) and may completely shut down glucose transporters (14), activated T cells often continue being glycolytically dependent even in well-oxygenated areas. A similar phenomenon occurs in cancers and is known as the Warburg effect (49–51). In this case, as both cancer and immune cells are heavily dependent on the glycolytic pathway for active proliferation, the competition may well become limiting for immune response in the tumor microenvironment, should cancer cells be more efficient at exploiting the glycolytic state. Also, while activated T cells cannot survive without adequate glucose supply and require both large amounts of glucose and receptor costimulation in order to be able to successfully take up the nutrients (11), tumor cells can enter quiescence for an extended period of time or even become "dormant" (52, 53) until conditions improve. Moreover, as tumor cells can primarily accommodate self-perpetuation, whereas immune cells must carry out functions beyond their own survival, the shortfall may further disadvantage the ability of T cells to expand in the hostile environment (54).

These events in combination could allow cancer cells to effectively escape elimination by the adaptive immune response. Indeed, upregulation of glycolysis has been hypothesized to give a number of other selective advantages to cancer cells, including increased proliferative capacity that results from use of intermediate products of glycolysis for biosynthesis of nucleic acids (10) and increased invasiveness that results from pH-induced damage to somatic cells in the vicinity of the tumor (21, 55). It is also possible that tumors that maintain a tight control over pH in their vicinity and successfully compete with immune cells for necessary nutrients are those that are able to circumvent the antitumor immune response and progress to full malignancy. One could speculate that the established ability of low pH to selectively promote certain humoral and cellular immune activities is something that the

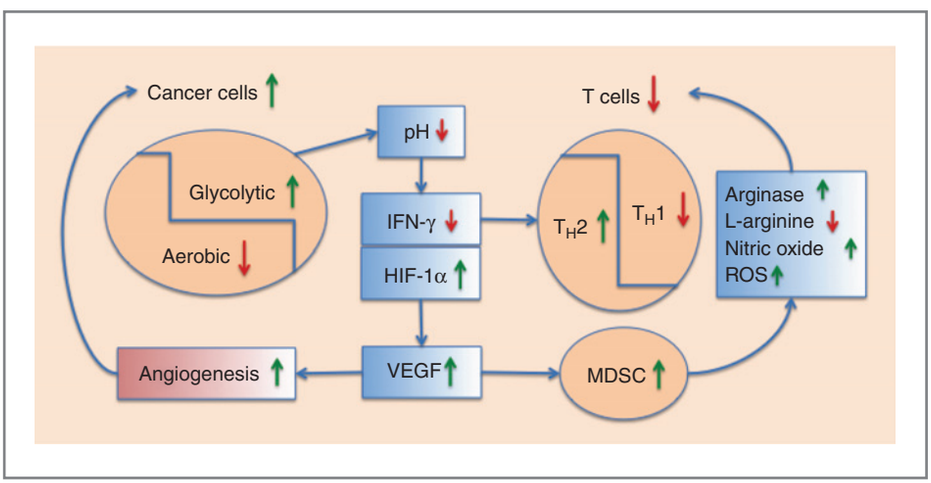

Figure 1. The cascade of effects resulting from upregulated glycolysis and consequent lowering of pH in the tumor microenvironment on the cells of the adaptive immune system.

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

tumor, through its own evolving bias to glycolytic acidosis, could exploit to promote the tumor population itself. In this way, low pH could, in the net, favor the tumor population in the tumor–immune competition and improve the likelihood of tumor escape.

These considerations may provide an explanation for the limited success of immunotherapy (56). Whether immunotherapy is targeted toward increasing the activity of antigen-presenting cells, such as DCs (57), or toward directly promoting the activity of cytotoxic T cells via adoptive transfer therapies (58), the conditions in the tumor microenvironment can be such that even the most potent immune cells will not accomplish their cytotoxic function. If, for instance, resources in the tumor microenvironment are limited due to upregulation of glycolysis by tumor cells, then activated cytotoxic T cells will not be able to undergo clonal expansion due to nutrient deprivation. Supporting evidence for this hypothesis comes from observations of an apparent synergistic effect when chemotherapy is administered concurrently with immunotherapy (59), possibly in part due to decreased competition for resources that follows from extensive mortality of cancer cells. Finding ways to manipulate the tumor microenvironment may thus be an important therapeutic step toward improvement of immune response, whether induced naturally or augmented through immunotherapy. More broadly, our increasing knowledge of the influences of altered glucose metabolism and increased immune invasion on tumor growth is revealing that two "hallmarks" of cancer thought to be distinct are in fact fundamentally linked, carrying vital implications for both biologic understanding and clinical approach.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: I. Kareva
Writing, review, and/or revision of the manuscript: I. Kareva, P. Hahnfeldt
Study supervision: P. Hahnfeldt

Grant Support

This research was supported by the Office of Science (BER), U.S. Department of Energy, under Award Number DE-SC0001434 (P. Hahnfeldt) and by the National Cancer Institute under Award Number U54CA149233 (Lynn Hlatky).

Received September 26, 2012; revised February 12, 2013; accepted February 12, 2013; published OnlineFirst February 19, 2013.

References

1. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–74.
2. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–55.
3. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 2009;27:451–83.
4. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 2012;12:253–68.
5. Ruffell B, DeNardo DG, Affara NI, Coussens LM. Lymphocytes in cancer development: polarization towards pro-tumor immunity. Cytokine Growth Factor Rev 2010;21:3–10.
6. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24–37.
7. Murphy K, Travers P, Walport M. Janeway's immunobiology. 7th ed. New York and London: Garland Science, Taylor & Francis Group; 2007.
8. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 2001;166:678–89.
9. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 2009;58:49–59.
10. Weinberg F, Chandel NS. Mitochondrial metabolism and cancer. Ann N Y Acad Sci 2009;1177:66–73.
11. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005;5:844–52.
12. Greiner EF, Guppy M, Brand K. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. J Biol Chem 1994;269:31484–90.
13. Brand K, Aichinger S, Forster S, Kupper S, Neumann B, Nürnberg W, et al. Cell-cycle-related metabolic and enzymatic events in proliferating rat thymocytes. Eur J Biochem 1988;172:695–702.
14. MacIver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. J Leukoc Biol 2008;84:949–57.
15. Ganapathy V, Thangaraju M, Prasad PD. Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. Pharmacol Ther 2009;121:29–40.
16. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood 2010;115:4742–9.
17. Semenza GL. Regulation of cancer cell metabolism by hypoxia-inducible factor 1. Semin Cancer Biol 2009;19:12–6.
18. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene 2010;29:625–34.
19. Harris AL. Hypoxia–a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.
20. Mekhail K, Gunaratnam L, Bonicalzi ME, Lee S. HIF activation by pH-dependent nucleolar sequestration of VHL. Nat Cell Biol 2004;6:642–7.
21. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4:891–9.
22. Norian LA, Rodriguez PC, O'Mara LA, Zabaleta J, Ochoa AC, Cella M, et al. Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. Cancer Res 2009;69:3086–94.
23. Harari O, Liao JK. Inhibition of MHC II gene transcription by nitric oxide and antioxidants. Curr Pharm Des 2004;10:893–8.
24. Bogdan C. Regulation of lymphocytes by nitric oxide. Methods Mol Biol 2011;677:375–93.
25. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. J Immunol 2004;172:989–99. Erratum in: J Immunol. 2004 Apr 1;172:4647.
26. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 2010;207:2439–53.
27. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, et al. Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. Cancer Res 2010;70:7465–75.

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

28. Kim WY, Kaelin WG. Role of VHL gene mutation in human cancer. J Clin Oncol 2004;22:4991–5004.
29. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol 2007;96:41–101.
30. Hu X, Ivashkiv LB. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. Immunity 2009;31:539–50.
31. Enzler T, Gillessen S, Manis JP, Ferguson D, Fleming J, Alt FW, Mihm M, Dranoff G. Deficiencies of GM-CSF and interferon gamma link inflammation and cancer. J Exp Med 2003;197:1213–9.
32. Raes G, Beschin A, Ghassabeh GH, De Baetselier P. Alternatively activated macrophages in protozoan infections. Curr Opin Immunol 2007;19:454–9.
33. Nathan I, Groopman JE, Quan SG, Bersch N, Golde DW. Immune (gamma) interferon produced by a human T-lymphoblast cell line. Nature 1981;292:842–4.
34. Barth RJ Jr, Mulé JJ, Spiess PJ, Rosenberg SA. Interferon gamma and tumor necrosis factor have a role in tumor regressions mediated by murine CD8+ tumor-infiltrating lymphocytes. J Exp Med 1991;173:647–58.
35. Heming TA, Davé SK, Tuazon DM, Chopra AK, Peterson JW, Bidani A. Effects of extracellular pH on tumour necrosis factor-alpha production by resident alveolar macrophages. Clin Sci (Lond) 2001;101:267–74.
36. Lardner A. The effects of extracellular pH on immune function. J Leukoc Biol 2001;69:522–30.
37. López DH, Trevani AS, Salamone G, Andonegui G, Raiden S, Giordano M, et al. Acidic pH increases the avidity of FcgammaR for immune complexes. Immunology 1999;98:450–5.
38. Hammer CH, Hänsch G, Gresham HD, Shin ML. Activation of the fifth and sixth components of the human complement system: C6-dependent cleavage of C5 in acid and the formation of a bimolecular lytic complex, C5b,6a. J Immunol 1983;131:892–8.
39. Fishelson Z, Horstmann RD, Müller-Eberhard HJ. Regulation of the alternative pathway of complement by pH. J Immunol 1987;138:3392–5.
40. Emeis M, Sonntag J, Willam C, Strauss E, Walka MM, Obladen M. Acidosis activates complement system *in vitro*. Mediators Inflamm 1998;7:417–20.
41. Sonntag J, Emeis M, Strauss E, Obladen M. *In vitro* activation of complement and contact system by lactic acidosis. Mediators Inflamm 1998;7:49–51.
42. Martínez D, Vermeulen M, Trevani A, Ceballos A, Sabatté J, Gamberale R, et al. Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. J Immunol 2006;176:1163–71.
43. Zhang J, Koh J, Lu J, Thiel S, Leong BS, Sethi S, et al. Local inflammation induces complement crosstalk which amplifies the antimicrobial response. PLoS Pathog 2009;5:e1000282.
44. Trevani AS, Andonegui G, Giordano M, López DH, Gamberale R, Minucci F, et al. Extracellular acidification induces human neutrophil activation. J Immunol 1999;162:4849–57.
45. Tong J, Wu WN, Kong X, Wu PF, Tian L, Du W, et al. Acid-sensing ion channels contribute to the effect of acidosis on the function of dendritic cells. J Immunol 2011;186:3686–92.
46. Vermeulen M, Giordano M, Trevani AS, Sedlik C, Gamberale R, Fernández-Calotti P, et al. Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells. J Immunol 2004;172:3196–204.
47. Martínez D, Vermeulen M, von Euw E, Sabatté J, Maggíni J, Ceballos A, et al. Extracellular acidosis triggers the maturation of human dendritic cells and the production of IL-12. J Immunol 2007;179:1950–9.
48. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
49. Kim JW, Dang CV. Cancer's molecular sweet tooth and the Warburg effect. Cancer Res 2006;66:8927–30.
50. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029–33.
51. Warburg O. On the origin of cancer cells. Science 1956;123:309–14.
52. Almog N. Molecular mechanisms underlying tumor dormancy. Cancer Lett 2010;294:139–46.
53. Bragado P, Sosa MS, Keely P, Condeelis J, Aguirre-Ghiso JA. Microenvironments dictating tumor cell dormancy. Recent Results Cancer Res 2012;195:25–39.
54. Thompson ED, Enriquez HL, Fu YX, Engelhard VH. Tumor masses support naive T cell infiltration, activation, and differentiation into effectors. J Exp Med 2010;207:1791–804.
55. Gillies RJ, Robey I, Gatenby RA. Causes and consequences of increased glucose metabolism of cancers. J Nucl Med 2008;49 Suppl 2:24S–42S.
56. Lesterhuis WJ, Haanen JB, Punt CJ. Cancer immunotherapy–revisited. Nat Rev Drug Discov 2011;10:591–600.
57. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008;29:372–83.
58. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126–9.
59. Nowak AK, Robinson BW, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63:4490–6.

Published OnlineFirst February 19, 2013; DOI: 10.1158/0008-5472.CAN-12-3696

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

The Emerging "Hallmarks" of Metabolic Reprogramming and Immune Evasion: Distinct or Linked?

Irina Kareva and Philip Hahnfeldt

Cancer Res 2013;73:2737-2742. Published OnlineFirst February 19, 2013.