Cancer Metastasis Rev (2007) 26:273–279
DOI 10.1007/s10555-007-9054-2

Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues

D. Lukashev · A. Ohta · M. Sitkovsky

Published online: 3 April 2007

Abstract The evolutionarily selected tissue-protecting mechanisms are likely to be triggered by an event of universal significance for all surrounding cells. Such an event could be damage to blood vessels, which would result in local tissue hypoxia. It is now recognized that tissue hypoxia can initiate the tissue-protecting mechanism mediated by at least two different biochemical pathways. The central message of this review is that tumor cells are protected from immune damage in hypoxic and immunosuppressive tumor microenvironments due to the inactivation of anti-tumor T cells by the combined action of these two hypoxia-driven mechanisms. Firstly, tumor hypoxia-produced extracellular adenosine inhibits anti-tumor T cells via their G_s-protein-coupled and cAMP-elevating A2A and A2B adenosine receptors (A2AR/A2BR). Levels of extracellular adenosine are increased in tumor microenvironments due to the changes in activities of enzymes involved in adenosine metabolism. Secondly, TCR-activated and/or tumor hypoxia-exposed anti-tumor T cells may be inhibited in tumor microenvironments by Hypoxia-inducible Factor 1α (HIF-1α) Hence, HIF-1α activity in T cells may contribute to the tumor-protecting immunosuppressive effects of tumor hypoxia. Here, we summarize the data that support the view that protection of hypoxic cancerous tissues from anti-tumor T cells is mediated by the same mechanism that protects normal tissues from the excessive collateral damage by overactive immune cells during acute inflammation.

D. Lukashev · A. Ohta · M. Sitkovsky (✉)
New England Inflammation and Tissue Protection Institute,
Northeastern University,
360 Huntington Avenue, 113 Mugar,
Boston, MA 02115, USA
e-mail: m.sitkovsky@neu.edu

Keywords Adenosine · Cancer · Hypoxia · Immunotherapy · T cells

Abbreviations

A2AR	adenosine A2A receptor
A2BR	adenosine A2B receptor
ADA	adenosine deaminase
AK	adenosine kinase
ENT	equilibrative nucleoside transporter
HIF-1α	hypoxia-inducible factor 1α
NT	5′-AMP nucleotidase
TCR	T-cell receptor

1 The physiological mechanism that prevents excessive collateral tissue damage during immune response

The complete eradication of pathogens depends on a prompt and efficient immune response. Inflammatory processes are initiated by immune cells through secretion of various pro-inflammatory cytokines and chemokines, which results in activation and migration of myeloid cells and lymphocytes to the area of inflammation [1, 2]. The combined pro-inflammatory actions of cells of the innate and adaptive immune systems lead to the damage to bacteria or infected cells. However, immune cells not only destroy pathogens but might also cause collateral injury to normal tissues. It was recently explained that the collateral damage by immune cells is limited by the 'danger-sensing' physiological mechanism [3], which results in the tissue-protecting negative feedback inhibition of overactive immune cells. This phenomenon may explain the surprisingly rare post-inflammatory complications in infected patients.

This crucial anti-inflammatory mechanism functions by engaging the adenosine receptor A2A (A2AR) on the surface of immune cells [4–7]. It was demonstrated that

extracellular adenosine that is accumulated in inflamed areas signals through the A2AR leading to the increase of immunosuppressive cAMP [3, 8]. As a result, activation of adenosine->A2AR pathway leads to down-regulation of pro-inflammatory functions of immune cells and to subsequent resolution of tissue-destructing inflammation [7].

Four different adenosine receptors are currently characterized: G_i-protein-coupled A1 and A3 receptors, and G_s-protein-coupled A2A and A2B receptors [6, 9, 10]. It was shown that T cells predominantly express A2AR and A2BR [9, 11–15]. The signaling through A2AR or A2BR in T cells results in elevation of cAMP levels and consequent inhibition of TCR-triggered activation of T cells [14, 16–18] and their effector functions, including proliferation, expansion and secretion of cytokines such as IFN-γ and TNF-α [13, 18–20].

2 The problem of cancer immunotherapy: some areas of cancerous tissues are protected from anti-tumor T cells

The studies of tissue protection from activated immune cells have immediate implications for the understanding of how tumors are protected from anti-tumor T cells. The critical role of T cells in cancer immunosurveillance was shown in a number of mouse models [21] and human patients studies [12, 22–30]. It was proven that the presence of T lymphocytes inside of solid tumors is a predictive factor for improved clinical outcome during esophageal carcinoma [23], colorectal cancer [22], and ovarian cancer [24, 25]. Recently, some advances were reported in adoptive T cell therapy against several forms of cancer [31, 32] with improved development of endogenous anti-tumor CD8+T lymphocytes [12, 27–30, 33–36] and NK and NKT cells [37, 38].

However, the use of T-cell-based immunotherapy appears to be hindered by "hostile" immunosuppressive tumor microenvironment that prevents tumor destruction by T cells [39–41]. For example, T-cell-mediated tumor rejection is rare despite that T lymphocytes can recognize antigens expressed by melanoma, and regardless of the massive influx of tumor antigen-specific T cells to the tumor site [42]. The co-existence of tumors and anti-tumor immune cells ("Hellstrom Paradox") [26, 39, 41, 43–45] has been a challenging problem for a long time.

This paradox prompted speculations that tumor microenvironment *in vivo* may prevent anti-tumor CD8+ T cells from eliminating tumor. Thus, while some of the obstructions to the successful adoptive T cell transfer therapy may be due to the activity of suppressor T cells or anti-inflammatory cytokines [46, 47], other data indicate that the tumor microenvironment itself is capable of suppressing T-cell activity [42, 43].

Recent studies of immunosuppressive functions of adenosine receptors in immune cells during inflammation [4, 5, 48] suggest that extracellular adenosine may provide cancerous tissues protection from destruction by cytolytic anti-tumor T cells. The evidence that normal tissues in inflamed, and consequently hypoxic areas, protect themselves from immune damage by triggering A2A receptors on immune cells prompted the hypothesis that hypoxic tumors may use the same physiological mechanism to impede the attack by anti-tumor T cells (Fig. 1).

Indeed, it was demonstrated that extracellular adenosine produced by hypoxic tumors can prevent anti-tumor T cells from the successful destruction of tumors through the activity of A2A adenosine receptor on T cells [49]. It was shown that the absence of A2AR results in increased eradication of tumors in A2A-deficient mice. Moreover, significant improvement in tumor destruction can be achieved by the use of A2AR antagonists or by specific siRNA [49]. These data put forward the possibility of the development of new cancer immunotherapies using the strategy of targeting hypoxia-adenosine-A2A signaling pathway to prevent inhibition of anti-tumor T lymphocytes in hypoxic tumor microenvironments.

3 Hypoxia-adenosine-A2AR pathway in acute inflammation

Important clues for understanding cancerous tissue protection from anti-tumor T cells have been provided by recent insights into mechanisms of normal tissue protection from overactive immune cells through A2A adenosine receptor [4, 50, 51]. It was demonstrated that the same physiological mechanism that has evolved to function in damaged and hypoxic normal tissues [50, 51] also may be involved in indiscriminately protecting hypoxic cancerous tissue [49].

The physiological mechanism of protection of normal tissues from overactive immune cells by extracellular adenosine may be triggered by local tissue hypoxia that follows the excessive collateral immune damage to endothelial cells

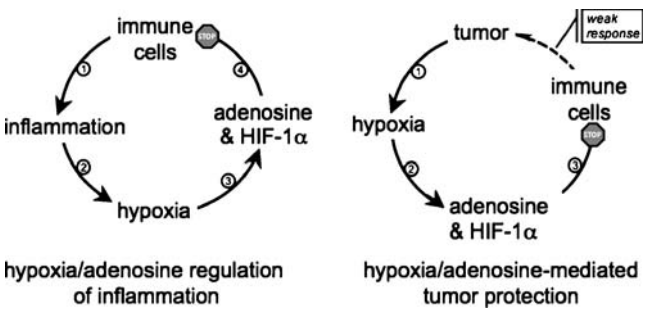

Fig. 1 Role of hypoxia-adenosine pathway in protection of inflamed and cancerous tissues

and microcirculation with ensuing interruption of normal blood and oxygen supply [7, 50]. The collateral tissue injury results in extracellular environment changes in the site of inflammation [52]. The blood supply in the inflamed areas is regularly blocked by clogging macrophages, which results in local tissue hypoxia [53, 54]. Likewise, increased tissue adenosine levels are commonly associated with hypoxia, and given the anti-inflammatory properties of adenosine, it was suggested that adenosine production via adenine nucleotide metabolism at the vascular surface triggers an endogenous anti-inflammatory response during hypoxia [52, 55]. It is well-established that inflamed and cancerous tissues are often characterized by low oxygen tension due to interruption of oxygen delivery through capillaries [56–59]. Accordingly, the levels of extracellular adenosine are shown to be high in hypoxic areas of inflamed tissues and solid tumors [49].

It is well established that hypoxia dramatically alters cellular metabolism, which results in accumulation of adenosine in the extracellular environment [60]. Sufficiently high levels of extracellular adenosine can trigger signaling by A2AR and/or A2BR on the surface of surrounding cells, including activated T cells, which culminates in the inhibition of overactive immune cells in a negative feedback manner [4, 7, 50, 51].

The hypoxia-induced increase of extracellular adenosine levels can be explained by changes in activities of several enzymes involved in adenosine metabolism (Fig. 2). The levels of intracellular adenosine are determined by activities of several key enzymes (reviewed in [60–62]):

1. *-5′-AMP nucleotidase (NT)* can promote adenosine formation from adenosine monophosphate (AMP);
2. *-adenosine kinase (AK)* can conversely re-phosphorylate adenosine into AMP;
3. *-adenosyl-homocysteine hydrolase* can reversibly convert adenosine into S-adenosyl-homocysteine
4. *-adenosine deaminase (ADA)* can transform adenosine into inosine;
5. *-equilibrative nucleoside transporters (ENT)* can transport adenosine through membrane in both directions [63].

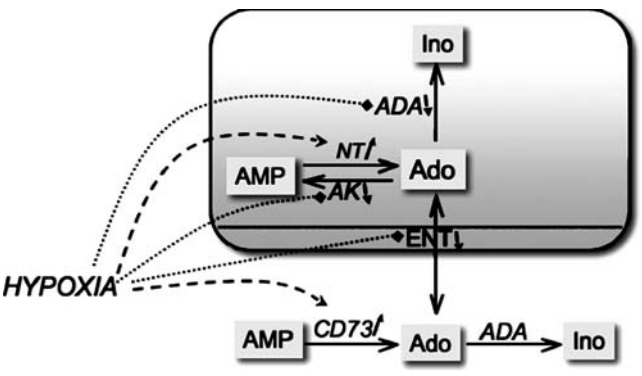

Fig. 2 Hypoxic regulation of adenosine metabolism. *Ado*, adenosine; *ADA*, adenosine deaminase; *AK*, adenosine kinase; *AMP*, adenosine monophosphate; *Ino*, inosine; *ENT*, equilibrative nucleoside transporter; *NT*, 5′-AMP nucleotidase; *CD73*, ecto-5′-AMP nucleotidase

The levels of extracellular adenosine are determined by *adenosine flux* through the membrane [64], and by conversion from extracellular AMP through activity of *ecto-5′-AMP nucleotidase (CD73)* [65]. The extracellular adenosine can be converted then to inosine by *ecto-adenosine deaminase* [66] A number of studies have demonstrated that enzymes involved in adenosine metabolism can be affected by hypoxia. It was shown that adenosine formation is proportional to the AMP substrate concentration and that adenosine kinase activity is decreased during hypoxia [67]. The inhibition of adenosine kinase results in the shunting of intracellular adenosine from the salvage pathway to extracellular release. Due to normal high turnover of the AMP-adenosine metabolic cycle, the hypoxia-induced inhibition of adenosine kinase causes the amplification of small changes in free AMP into a major rise in adenosine. While not yet directly tested in hypoxic tumors, this mechanism plays an important role in the high sensitivity of the cardiac adenosine system to impaired oxygenation.

It is known that intravascular nucleotides released by inflammatory cells undergo phosphohydrolysis via hypoxia-induced CD39 ectoapyrase, which converts ATP and ADP to AMP, and by CD73 ecto-5′-nucleotidase that converts AMP to adenosine [60]. Among the processes that are affected by cell surface ecto-nucleotidase CD39/ecto-nucleoside triphosphate diphosphohydrolase-type-1 (ENTPD1) [68] are the endothelial cell, leukocyte and platelet responses to extracellular nucleotides during thrombosis and vascular inflammation [69, 70]. The dramatic increase in CD39 ecto-ATPase activity above the level of normal melanocytes was demonstrated in differentiated melanomas [71]. It was suggested that since CD39 is known to regulate homotypic adhesion and may affect the disaggregation step, overexpression of CD39 may enable tumor cells to reduce contacts with T lymphocytes and escape from immunological recognition. Recent studies of CD39- and CD73-deficient animals concluded that CD39 and CD73 serve as critical control points for endogenous adenosine generation and implicate this pathway as an innate mechanism to attenuate excessive polymorphonuclear leukocyte accumulation in tissues [55].

Hypoxia can inhibit activities of adenosine kinase and adenosine deaminase, while increasing the activity of 5′-nucleotidase [67, 72], resulting in increased levels of intracellular adenosine. Hypoxia also leads to the reduced expression of equilibrative nucleoside transporters, which

leads to decreased adenosine uptake and consequent extracellular accumulation of adenosine, accompanied by hypoxia-induced increase in adenosine release [64].

Taken together, these data indicate that levels of extracellular adenosine should be dramatically increased in hypoxic areas. As a result, accumulated extracellular adenosine can trigger G_s-coupled A2A adenosine receptors of activated immune cells [4], which will lead to the inhibition of immune functions in hypoxic conditions such as in cancerous tissues. Therefore, one can expect that immune cells infiltrating into hypoxic solid tumors, will encounter high concentrations of immunosuppressive adenosine in addition to the unfavorable hypoxic environment. Importantly, the generation of adenosine from extracellular AMP by ecto-5′-nucleotidase (CD73) is upregulated by hypoxia due to the induction of CD73 expression by hypoxia-inducible transcriptional factor 1 (HIF-1) [73].

4 Effect of hypoxia and hypoxia-inducible factor 1α on anti-tumor T cells

It is accepted that hypoxic conditions are unlikely to be conducive for immune cell functioning [52, 74]. Immune cells cannot avoid hypoxic and anoxic tissue microenvironments in order to fulfill their immunosurveillance function. Therefore, they need to possess adaptive metabolic mechanisms that allow them to generate energy for survival in conditions of oxygen deficiency and be capable to execute reactive oxygen species-dependent cytotoxic functions [74–76]. Among such metabolic adaptations is the transition of cellular energy production from oxygen-dependent process of oxidative phosphorylation to anaerobic glycolysis [52]. Indeed, immune cells can successfully use glycolysis as their energy source, and it was demonstrated that leukocytes rely on glycolysis as their main strategy of ATP-synthesis [77, 78]. Moreover, even in normoxic conditions myeloid cells prefer glycolysis rather than oxidative phosphorylation [79], while T cells can switch to glycolysis after activation [80].

It is recognized that hypoxia-inducible transcriptional factor 1 (HIF-1) is a key factor in the cellular adaptation to hypoxic conditions, including cell survival, angiogenesis, and the switch to glycolysis [81]. It was shown that overexpression of Hypoxia-inducible factor 1α in human cancers represent poor prognosis for the eradication of the tumor [82]. This stimulated the development of pharmacological agents capable to inhibit HIF-1α in tumors [83].

It is generally accepted that HIF-1α subunit of HIF-1 dimer is tightly regulated by hypoxia via ubiquitin-mediated degradation mechanism [84]. However, HIF-1α mRNA expression and protein levels in T cells can be enhanced by non-hypoxic stimuli that include TCR- and PI3K-mediated pathways [85–87]. Since HIF-1α is a crucial factor involved in neonatal vascularization, the studies of its gene-deficiency in animals were hindered [88]. Recently, mice with tissue-specific deletions of HIF-1α were developed in order to bypass the embryonic lethality setback. Studies of myeloid- and lymphoid-specific HIF-1α knockout mice demonstrated that HIF-1α may have different functions in various types of immune cells [52]. The conditional deletion of HIF-1α gene in myeloid cells was accomplished by using the myeloid-cell-specific Cre-recombinase expression system, which demonstrated that HIF-1α is critically required for glycolytic energy production by myeloid cells at both normoxic and hypoxic conditions [89]. HIF-1α-deficient neutrophils and macrophages were shown to have impaired metabolism and inhibited inflammatory response [89]. Consequently, HIF-1α was shown to be essential for infiltration and pathogen destruction by myeloid cells [89].

Studies of HIF-1α-deficiency in T cells revealed that HIF-1α not only plays crucial role in oxygen homeostasis, but may also serve as a negative regulator of the adaptive immune response. The role of HIF-1α in lymphoid cells was first tested using the RAG-2-blastocyst complementation system [90]. It was found that chimeric mice with the deletion of HIF-1α in T- and B-lymphocytes, showed increased autoimmune tissue damage accompanied by abnormal maturation of B cells [90]. Recently, mice with T-cell-specific deletion of HIF-1α were created using Lck-Cre transgenic mice [91]. In direct opposition to the myeloid cells, it was shown that absence of HIF-1α in T cells leads to the upregulation of T-cell functions [91]. These observations suggested that while HIF-1α is essential for macrophage metabolism and pro-inflammatory functions, it may play an inhibitory role in T-cell functioning. Therefore, together with A2A adenosine receptor, HIF-1α may represent part of the anti-inflammatory mechanism of attenuating T-cell response (Fig. 1). This may also indicate that while hypoxia/HIF-1α- and adenosine/A2AR-mediated pathways play important role in protection of normal tissues from collateral immune damage, tumors may also "hijack" these mechanisms for protection from immune system (Fig. 1).

The TCR activation of T lymphocytes leads to "immediate-early response gene"-like transcriptional upregulation of the shorter alternatively-spliced isoform I.1 of HIF-1α [85]. It was recently shown that even though the levels of this short isoform were significantly less than the full-length HIF-1α, genetic deletion of I.1 isoform resulted in significant increase in TCR-induced T-cell response [91]. These data suggest that "immediate-early response gene" short isoform I.1 of HIF-1α is disproportionately important in attenuation of activated T cells in a delayed negative feed-back manner.

Taken together, the recent studies support the view that HIF-1α is a negative regulator of T-cell functions [91, 92]

and suggest that HIF-1α may also play a role in inhibition of anti-tumor T cells. Considering previous observations that HIF-1α expression is induced by T-cell activation [85, 87], it is likely that HIF-1α represents a part of the negative feed-back loop mechanism that leads to attenuation of activated T cells. This HIF-1α-mediated anti-inflammatory pathway may be complimentary to the immunosuppressive mechanism of tissue protection from excessive immune damage that is mediated by A2A adenosine receptor and by hypoxia-induced extracellular adenosine [52].

5 Conclusion

Direct tumor rejection studies and modeling of T-cell-mediated immunity in *in vitro* assays strongly suggest that the hypoxia-stabilized and TCR-activation-induced HIF-1α may cooperate in inhibiting anti-tumor T cells in tumor microenvironment. Therefore, new therapies may involve the application of antagonists of A2A receptor and HIF-1α inhibitors to prevent the inhibition of T cells and lead to more efficient elimination of tumors by T lymphocytes in novel cancer immunotherapy protocols.

Acknowledgements Authors thank Stephen Hatfield for the help in preparation of the manuscript. This work was supported by NIH Grants 1 R01 CA112561-01, 1 R01 CA111985-01A2, and 1 R21 AT002788-01A1.

References

1. Rosenberg, H. F., & Gallin, J. I. (1999). Inflammation. In W. E. Paul (Ed.), *Fundamental immunology* (pp. 1051–1062). Philadelphia: Lippincott-Raven.
2. Holloway, A. F., Rao, S., & Shannon, M. F. (2002). Regulation of cytokine gene transcription in the immune system. *Molecular Immunology, 38*, 567–80.
3. Sitkovsky, M. V., & Ohta, A. (2005). The 'danger' sensors that STOP the immune response: The A2 adenosine receptors? *Trends in Immunology, 26*, 299–304.
4. Ohta, A., & Sitkovsky, M. (2001). Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature, 414*, 916–920.
5. Lukashev, D., Ohta, A., Apasov, S., Chen, J. F., & Sitkovsky, M. (2004). Cutting edge: Physiologic attenuation of proinflammatory transcription by the Gs protein-coupled A2A adenosine receptor in vivo. *Journal of Immunology, 173*, 21–24.
6. Linden, J. (2001). Molecular approach to adenosine receptors: Receptor-mediated mechanisms of tissue protection. *Annual Review of Pharmacology and Toxicology, 41*, 775–787.
7. Sitkovsky, M. V., Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., et al. (2004). Thiel M: Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annual Review of Immunology, 22*, 657–682.
8. Sattin, A., Rall, T. W. (1970). The effect of adenosine and adenine nucleotides on the cyclic adenosine 3', 5'-phosphate content of guinea pig cerebral cortex slices. *Molecular Pharmacology, 6*, 13–23.
9. Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Klotz, K.-N., Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological Reviews, 53*, 527–5522.
10. Fredholm, B. B., Battig, K., Holmen, J., Nehlig, A., Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*, 83–133.
11. Lukashev, D. E., Smith, P. T., Caldwell, C. C., Ohta, A., Apasov, S. G., Sitkovsky, M. V. (2003). Analysis of A2a receptor-deficient mice reveals no significant compensatory increases in the expression of A2b, A1, and A3 adenosine receptors in lymphoid organs. *Biochemical Pharmacology, 65*, 2081–2090.
12. Chambers, C. A., Kuhns, M. S., Egen, J. G., & Allison, J. P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annual Review of Immunology, 19*, 565–594.
13. Koshiba, M., Kojima, H., Huang, S., Apasov, S., & Sitkovsky, M. V. (1997). Memory of extracellular adenosine/A2a purinergic receptor-mediated signalling in murine T cells. *Journal of Biological Chemistry, 272*, 25881–25889.
14. Koshiba, M., Rosin, D. L., Hayashi, N., Linden, J., & Sitkovsky, M. V. (1999). Patterns of A2A extracellular adenosine receptor expression in different functional subsets of human peripheral T cells. Flow cytometry studies with anti-A2A receptor monoclonal antibodies. *Molecular Pharmacology, 55*, 614–624.
15. Huang, S., Koshiba, M., Apasov, S., & Sitkovsky, M. (1997). Role of A2a adenosine receptor-mediated signaling in inhibition of T cell activation and expansion. *Blood, 90*, 1600–1610.
16. Takayama, H., & Sitkovsky, M. V. (1988). Potential use of antagonists of cAMP-dependent protein kinase to block inhibition and modulate T-cell receptor-triggered activation of cytotoxic T-lymphocytes. *Journal of Pharmaceutical Sciences, 78*, 8–10.
17. Sugiyama, H., Chen, P., Hunter, M., Taffs, R., & Sitkovsky, M. (1992). The dual role of the cAMP-dependent protein kinase C alpha subunit in T-cell receptor-triggered T-lymphocytes effector functions. *Journal of Biological Chemistry, 267*, 25256–25263.
18. Torgersen, K. M., Vang, T., Abrahamsen, H., Yaqub, S., & Tasken, K. (2002). Molecular mechanisms for protein kinase A-mediated modulation of immune function. *Cell Signal, 14*, 1–9.
19. Sun, H., Gutierrez, P., Jackson, M. J., Kundu, N., & Fulton, A. M. (2000). Essential role of nitric oxide and interferon-gamma for tumor immunotherapy with interleukin-10. *Journal of Immunotherapy, 23*, 208–214.
20. Poehlein, C. H., Hu, H. M., Yamada, J., Assmann, I., Alvord, W. G., Urba, W. J., et al. (2003). TNF plays an essential role in tumor regression after adoptive transfer of perforin/IFN-gamma double knockout effector T cells. *Journal of Immunology, 170*, 2004–2013.
21. Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., & Old, L. J., et al. (2001). IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature, 410*, 1107–1111.
22. Naito, Y., Saito, K., Shiiba, K., Ohuchi, A., Saigenji, K., & Nagura, H., et al. (1998). CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Research, 58*, 3491–3494.
23. Schumacher, K., Haensch, W., Roefzaad, C., & Schlag, P. M. (2001). Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. *Cancer Research, 61*, 3932–3936.
24. Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *New England Journal of Medicine, 348*, 203–213.
25. Nagorsen, D., Scheibenbogen, C., Marincola, F., Letsch, A., & Keilholz, U. (2003). Natural T cell immunity against cancer. *Clinical Cancer Research, 9*, 4296–4303.

26. Rosenberg, S. A. (2001). Progress in the development of immunotherapy for the treatment of patients with cancer. *Journal of Internal Medicine, 250*, 462–475.
27. Melief, C. J., Van Der Burg, S. H., Toes, R. E., Ossendorp, F., & Offringa, R. (2002). Effective therapeutic anticancer vaccines based on precision guiding of cytolytic T lymphocytes. *Immunological Reviews, 188*, 177–182.
28. Hurwitz, A. A., Yanover, P., Markowitz, M., Allison, J. P., & Kwon, E. D. (2003). Prostate cancer: Advances in immunotherapy. *Bio Drugs, 17*, 131–138.
29. Ribas, A., Butterfield, L. H., Glaspy, J. A., & Economou, J. S. (2003). Current developments in cancer vaccines and cellular immunotherapy. *Journal of Clinical Oncology, 21*, 2415–2432.
30. Wang, X. Y., Kazim, L., Repasky, E. A., & Subjeck, J. R. (2003). Immunization with tumor-derived ER chaperone grp170 elicits tumor-specific CD8+ T-cell responses and reduces pulmonary metastatic disease. *International Journal of Cancer, 105*, 226–231.
31. Morgan, R. A., Dudley, M. E., Wunderlich, J. R., Hughes, M. S., Yang, J. C., & Sherry, R. M., et al. (2006). Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science, 314*, 126–129.
32. Gattinoni, L., Powell, D. J., Jr., Rosenberg, S. A., & Restifo, N. P. (2006). Adoptive immunotherapy for cancer: building on success. *Nature Reviews. Immunology, 6*, 383–393.
33. Chouhury, A., Mosolits, S., Kokhaei, P., Hansson, L., Palma, M., & Mellstedt, H. (2006). Clinical results of vaccine therapy for cancer: Learning from history for improving the future. *Advances in Cancer Research, 95*, 147–202.
34. Hodi, F. S. (2006). Dranoff G, combinatorial cancer immunotherapy. *Advances in Immunology, 90*, 341–368.
35. Elia, L., Aurisicchio, L., Fcciabene, A., Giannetti, P., Ciliberto, G., La Monica, N., et al. (2007). CD4(+)CD25(+) regulatory T-cell-inactivation in combination with adenovirus vaccines enhances T-cell responses and protects mice from tumor challenge. *Cancer Gene Therapy,* 201–210.
36. Borges, V. F., Kufe, D., & Avigan, D. E. (2002). Update on cancer vaccines. *Current Opinion in Gastroenterology, 18*, 723–731.
37. Woan, K., & Reddy, V. (2007). Potential therapeutic role of natural killer cells in cancer. *Expert Opin Biol Ther, 7*, 17–29.
38. van der Vliet, H. J., Balk, S. P., & Exley, M. A. (2006). Natural killer T cell-based cancer immunotherapy. *Clinical Cancer Research, 12*, 5921–5923.
39. Rabinovich, G. A., Gabrilovich, D., Sotomayor, E. M. (2007). Immunosuppressive strategies that are mediated by tumor cells. *Annual Review of Immunology, 25*, 267–296.
40. Leen, A. M., Rooney, C. M., & Foster, A. E. (2007). Improving T cell therapy for cancer. *Annual Review of Immunology, 25*, 243–265.
41. Frumento, G., Piazza, T., Di Carlo, E., & Ferrini, S. (2006). Targeting tumor-related immunosuppression for cancer immunotherapy. *Endocr Metab Immune Disord Drug Targets, 6*, 233–237.
42. Harlin, H., Kuna, T. V., Peterson, A. C., Meng, Y., & Gajewski, T. F. (2006). Tumor progression despite massive influx of activated CD8 (+) T cells in a patient with malignant melanoma ascites. *Cancer Immunology and Immunotherapy, 55*, 1185–1197.
43. Marincola, F. M., Jaffee, E. M., Hicklin, D. J., & Ferrone, S. (2000). Escape of human solid tumors from T-cell recognition: Molecular mechanisms and functional significance. *Advances in Immunology, 74*, 181–273.
44. Hellstrom, I., Hellstrom, K. E., Pierce, G. E., & Yang, J. P. (1968). Cellular and humoral immunity to different types of human neoplasms. *Nature, 220*, 1352–1354.
45. Hanson, H. L., Donermeyer, D. L., Ikeda, H., White, J. M., Shankaran, V., Old, L. J., et al. (2000). Eradication of established tumors by CD8+ T cell adoptive immunotherapy. *Immunity, 13*, 265–276.
46. Peng, L., Kjaergaard, J., Plautz, G. E., Awad, M., Drazba, J. A., Shu, S., et al. (2002). Tumor-induced L-selectinhigh suppressor T cells mediate potent effector T cell blockade and cause failure of otherwise curative adoptive immunotherapy. *Journal of Immunology, 169*, 4811–4821.
47. Kobie, J. J., Wu, R. S., Kurt, R. A., Lou, S., Adelman, M. K., Whitesell, L. J., et al. (2003). Transforming growth factor beta inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines. *Cancer Research, 63*, 1860–1864.
48. Lappas, C. M., Rieger, J. M., & Linden, J. (2005). A2A adenosine receptor induction inhibits IFN-gamma production in murine CD4+ T cells. *Journal of Immunology, 174*, 1073–1080.
49. Ohta, A., Gorelik, E., Prasad, S. J., Ronchese, F., Lukashev, D., Wong, M. K., et al. (2006). A2A adenosine receptor protects tumors from antitumor T cells. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 13132–13137.
50. Sitkovsky, M. V. (2003). Use of the A(2A) adenosine receptor as a physiological immunosuppressor and to engineer inflammation in vivo. *Biochemical Pharmacology, 65*, 493–501.
51. Thiel, M., Chouker, A., Ohta, A., Jackson, E., Caldwell, C., Smith, P., et al. (2005). Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. *PLoS Biol, 3*, e174.
52. Sitkovsky, M., & Lukashev, D. (2005). Regulation of immune cells by local-tissue oxygen tension: HIF1alpha and adenosine receptors. *Nature Reviews Immunology, 5*, 712–721.
53. Arnold, F., West, D., & Kumar, S. (1987). Wound healing: The effect of macrophage and tumour derived angiogenesis factors on skin graft vascularization. *British Journal of Experimental Pathology, 68*, 569–574.
54. Simmen, H. P., Battaglia, H., Giovanoli, P., & Blaser, J. (1994). Analysis of pH, pO2 and pCO2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. *Infection, 22*, 386–389.
55. Eltzschig, H. K., Thompson, L. F., Karhausen, J., Cotta, R. J., Ibla, J. C., Robson, S. C., et al. (2004). Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: Coordination by extracellular nucleotide metabolism. *Blood, 104*, 3986–3992.
56. Helmlinger, G., Yuan, F., Dellian, M., & Jain, R. K. (1997). Interstitial pH and pO2 gradients in solid tumors in vivo: High-resolution measurements reveal a lack of correlation. *Nat Med, 3*, 177–182.
57. Vaupel, P., Kalinowski, F., & Okunieff, P. (1989). Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. *Cancer Research, 49*, 6449–6465.
58. Vaupel, P., Thews, O., & Hoeckel, M. (2001). Treatment resistance of solid tumors: Role of hypoxia and anemia. *Medical Oncology, 18*, 243–259.
59. Harris, A. L. (2002). Hypoxia—a key regulatory factor in tumour growth. *Nature Reviews. Cancer, 2*, 38–47.
60. Gorlach, A. (2005). Control of adenosine transport by hypoxia. *Circulation Research, 97*, 1–3.
61. Arch, J. R., & Newsholme, E. A. (1978). Activities and some properties of 5'-nucleotidase, adenosine kinase and adenosine deaminase in tissues from vertebrates and invertebrates in relation to the control of the concentration and the physiological role of adenosine. *Biochemical Journal, 174*, 965–977.
62. Lukashev, D., Ohta, A., & Sitkovsky, M. (2004). Targeting hypoxia-A(2A) adenosine receptor-mediated mechanisms of tissue protection. *Drug Discovery Today, 9*, 403–409.
63. Griffith, D. A., & Jarvis, S. M. (1996). Nucleoside and nucleobase transport systems of mammalian cells. *Biochimica and Biophysica Acta, 1286*, 153–181.
64. Casanello, P., Torres, A., Sanhueza, F., Gonzalez, M., Farias, M., Gallardo, V., et al. (2005). Equilibrative nucleoside transporter 1 expression is downregulated by hypoxia in human umbilical vein endothelium. *Circulation Research, 97*, 16–24.

65. Zimmermann, H., & Braun, N. (1999). Ecto-nucleotidases-molecular structures, catalytic properties, and functional roles in the nervous system. *Progress in Brain Research, 120*, 371–385.
66. Cristalli, G., Costanzi, S., Lambertucci, C., Lupidi, G., Vittori, S., Volpini, R., et al. (2001). Adenosine deaminase: Functional implications and different classes of inhibitors. *Medical Research Reviews, 21*, 105–128.
67. Decking, U. K., Schlieper, G., Kroll, K., & Schrader, J. (1997). Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release. *Circulation Research, 81*, 154–164.
68. Kaczmarek, E., Koziak, K., Sevigny, J., Siegel, J. B., Anrather, J., Beaudoin, A. R., et al. (1996). Identification and characterization of CD39/vascular ATP diphosphohydrolase. *Journal of Biological Chemistry, 271*, 33116–33122.
69. Robson, S. C., Wu, Y., Sun, X., Knosalla, C., Dwyer, K., & Enjyoji, K. (2005). Ectonucleotidases of CD39 family modulate vascular inflammation and thrombosis in transplantation. *Seminars in Thrombosis and Hemostasis, 31*, 217–233.
70. Kaczmarek, E., Erb, L., Koziak, K., Jarzyna, R., Wink, M. R., Guckelberger, O., et al. (2005). Modulation of endothelial cell migration by extracellular nucleotides: Involvement of focal adhesion kinase and phosphatidylinositol 3-kinase-mediated pathways. *Thrombosis and Haemostasis, 93*, 735–742.
71. Dzhandzhugazyan, K. N., Kirkin, A. F., thor Straten, P., & Zeuthen, J. (1998). Ecto-ATP diphosphohydrolase/CD39 is overexpressed in differentiated human melanomas. *FEBS Letters, 430*, 227–230.
72. Kobayashi, S., Zimmermann, H., & Millhorn, D. E. (2000). Chronic hypoxia enhances adenosine release in rat PC12 cells by altering adenosine metabolism and membrane transport. *Journal of Neurochemistry, 74*, 621–632.
73. Synnestvedt, K., Furuta, G. T., Comerford, K. M., Louis, N., Karhausen, J., Eltzschig, H. K., et al. (2002). Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *Journal of Clinical Investigation, 110*, 993–1002.
74. Caldwell, C. C., Kojima, H., Lukashev, D., Armstrong, J., Farber, M., Apasov, S. G., et al. (2001). Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. *Journal of Immunology, 167*, 6140–6149.
75. Babior, B. M., Kipnes, R. S., & Curnutte, J. T. (1973). Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *Journal of Clinical Investigation, 52*, 741–744.
76. Nathan, C. F., Mercer-Smith, J. A., DeSantis, N. M., & Palladino, M. A. (1982). Role of oxygen in T cell-mediated cytolysis. *Journal of Immunology, 129*, 2164–2171.
77. McKeehan, W. L. (1982). Glycolysis, glutaminolysis and cell proliferation. *Cell Biol Int Rep, 6*, 635–650.
78. Levene, P. A., & Meyer, G. M. (1912) The action of leukocytes on glucose. *Journal of Biological Chemistry, 11*, 361–370.
79. Borregaard, N., & Herlin, T. (1982). Energy metabolism of human neutrophils during phagocytosis. *Journal of Clinical Investigation, 70*, 550–557.
80. Rathmell, J. C., Vander Heiden, M. G., Harris, M. H., Frauwirth, K. A., & Thompson, C. B. (2000). In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. *Molecular Cell, 6*, 683–692.
81. Semenza, G. L. (1999). Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annual Review of Cell and Developmental Biology, 15*, 551–578.
82. Semenza, G. L. (2002). HIF-1 and tumor progression: Pathophysiology and therapeutics. *Trends in Molecular Medicine, 8*, S62–S67.
83. Semenza, G. L. (2003). Targeting HIF-1 for cancer therapy. *Nature Reviews Cancer, 3*, 721–732.
84. Semenza, G. L. (2001). HIF-1, O(2), and the 3 PHDs: How animal cells signal hypoxia to the nucleus. *Cell, 107*, 1–3.
85. Lukashev, D. E., Caldwell, C. C., Ohta, A., Chen, P., & Sitkovsky, M. V. (2001). Differential regulation of two alternatively spliced isoforms of hypoxia-inducible factor-1 alpha in activated T lymphocytes. *Journal of Biological Chemistry, 276*, 48754–48763.
86. Thornton, R. D., Lane, P., Borghaei, R. C., Pease, E. A., Caro, J., & Mochan, E. (2000). Interleukin 1 induces hypoxia-inducible factor 1 in human gingival and synovial fibroblasts. *Biochemical Journal, 350*, 307–312.
87. Nakamura, H., Makino, Y., Okamoto, K., Poellinger, L., Ohnuma, K., Morimoto, C., et al. (2005). TCR engagement increases hypoxia-inducible factor-1 alpha protein synthesis via rapamycin-sensitive pathway under hypoxic conditions in human peripheral T cells. *Journal of Immunology, 174*, 7592–7599.
88. Iyer, N. V., Kotch, L. E., Agani, F., Leung, S. W., Laughner, E., Wenger, R. H., et al. (1998). Cellular and developmental control of O2 homestasis by hypoxia-inducible factor 1a. *Genes & Development, 12*, 149–162.
89. Cramer, T., Yamzanishi, Y., Clausen, B. E., Forster, I., Pawlinski, R., Mackman, N., et al. (2003). HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell, 112*, 645–657.
90. Kojima, H., Gu, H., Nomura, S., Caldwell, C. C., Kobata, T., Carmeliet, P., et al. (2002). Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha -deficient chimeric mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 2170–2174.
91. Lukashev, D., Klebanov, B., Kojima, H., Grinberg, A., Ohta, A., Berenfeld, L., et al. (2006). Cutting edge: Hypoxia-inducible factor 1alpha and its activation-inducible short isoform I.1 negatively regulate functions of CD4+ and CD8+ T lymphocytes. *Journal of Immunology, 177,* 4962–4965.
92. Neumann, A. K., Yang, J., Biju, M. P., Joseph, S. K., Johnson, R. S., Haase, V. H., et al. (2005). Hypoxia inducible factor 1 alpha regulates T cell receptor signal transduction. *Proceedings of the National Academy of Sciences of the United States of America, 102*, 17071–17076.