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REVIEW

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Adenosine A2A receptor antagonists: blockade of adenosinergic effects and T regulatory cells

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The intensity and duration of host responses are determined by protective mechanisms that control tissue injury by dampening down inflammation. Adenosine generation and consequent effects, mediated via A2A adenosine receptors (A2AR) on effector cells, play a critical role in the pathophysiological modulation of these responses *in vivo*. Adenosine is both released by hypoxic cells/tissues and is also generated from extracellular nucleotides by ecto-enzymes e.g. CD39 (ENTPD1) and CD73 that are expressed by the vasculature and immune cells, in particular by T regulatory cell. In general, these adenosinergic mechanisms minimize the extent of collateral damage to host tissues during the course of inflammatory reactions. However, induction of suppressive pathways might also cause escape of pathogens and permit dissemination. In addition, adenosinergic responses may inhibit immune responses while enhancing vascular angiogenic responses to malignant cells that promote tumor growth. Novel drugs that block A2AR-adenosinergic effects and/or adenosine generation have the potential to boost pathogen destruction and to selectively destroy malignant tissues. In the latter instance, future treatment modalities might include novel 'anti-adenosinergic' approaches that augment immune clearance of malignant cells and block permissive angiogenesis. This review addresses several possible pharmacological modalities to block adenosinergic pathways and speculates on their future application together with impacts on human disease.
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Abbreviations: A2AR, A2A adenosine receptors; ENTPD, ectonucleoside triphosphate diphosphohydrolase; Tregs, T regulatory cells

Introduction

Studies of the role of adenosine receptors in inflammation have a long history with important recent advances made in evaluation of the pharmacological anti-inflammatory effects of A2 adenosine receptor agonists and drugs that increase levels of intracellular adenosine (Cronstein and Weissmann, 1995; Sullivan and Linden, 1998; Cronstein *et al*., 1999; Fredholm *et al*., 2001; Linden, 2001; Jacobson and Gao, 2006). Among the unexpected was the realization that methotrexate, the gold standard for treatment for rheumatoid arthritis, may exert therapeutic effects *in vivo* by impacting upon anti-inflammatory A2A adenosine receptors (A2AR) (Cronstein, 2005). The development of selective A2AR agonists and their creative use not only allowed to extend their potential applications with many more diseases (Cassada *et al*., 2001; Hogan *et al*., 2001; Okusa *et al*., 2001; Feoktistov *et al*., 2002; Montesinos *et al*., 2002; Dhalla *et al*., 2003; Awad *et al*., 2006; Nemeth *et al*., 2007; Rivo *et al*., 2007), but also served as an elegant tool to identify specific cell population that might be responsible for ischemia reperfusion injury (Lappas *et al*., 2006).

It is crucial to point out that extracellular adenosine not only is released from hypoxic, injured tissues but is also generated from extracellular nucleotides by ectonucleotidases. CD39 (ENTPD1 (ectonucleoside triphosphate diphosphohydrolase-1), EC 3.6.1.5) is an ectonucleotidase that hydrolyses ATP/UTP and ADP/UDP to the respective nucleosides (Robson *et al*., 2005). AMP is then rapidly degraded to adenosine by soluble or membrane-bound 5′-nucleotidase, for example, CD73 (EC 3.1.3.5)-expressed lymphocytes (Resta *et al*., 1998).

Here, we review our long-term studies that led to the identification of the A2 adenosine receptor-mediated 'adenosinergic' pathway as critical in physiological regulation of immune response *in vivo* (Sitkovsky, 2004; Sitkovsky and Lukashev, 2005a). We will also discuss how A2AR agonists

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function to inhibit overactive immune cells *in vivo*. These advances have enabled a reciprocal approach to enhance the immune response through elimination of the immunosuppressive adenosinergic pathway (Ohta and Sitkovsky, 2001; Lukashev *et al*., 2004; Thiel *et al*., 2005; Ohta *et al*., 2006; See Figure 1).

The novel opportunities for the therapeutic use of A2 adenosine receptor antagonists in cancer immunotherapy and vaccines (Ohta *et al*., 2006) can be further put into context by recent important demonstrations. T regulatory cells (Treg) express CD39 and CD73 ectonucleotidases, involving the immunosuppressive adenosinergic pathway in their mode of action (Kobie *et al*., 2006; Deaglio *et al*., 2007). CD39 ectoenzymatic function is also expressed by dendritic cells and is involved in the recruitment, activation and polarization of naïve T cells, as shown in *Cd39*-null mice (Mizumoto *et al*., 2002). These studies suggest that drugs such as A2AR antagonists might also prevent immunosuppression mediated through the generation of adenosine by Tregs and further strengthen the rationale of anti-adenosinergic strategies with A2AR antagonists (Ohta *et al*., 2006) to enhance immune responses.

Extracellular adenosine and genetic evidence for the critical role of A2A adenosine receptor in control of inflammation *in vivo*

The investigations reviewed here have been motivated by the need to address unresolved issues in cancer immunotherapy (Hellstrom *et al*., 1968). It would be very attractive if one could facilitate the destruction of cancerous tissues by the patient's own antitumour T cells. However, it is well established that T cells recognize and destroy tumour cells *in vitro*, but fail to do so *in vivo*. The long sought-after explanation of the coexistence of tumours and antitumour immune cells in a cancer patient or in a mouse ('Hellstrom Paradox') (Hellstrom *et al*., 1968; Hanson *et al*., 2000; Marincola *et al*., 2000; Rosenberg, 2001; Frumento *et al*., 2006; Leen *et al*., 2007; Rabinovich *et al*., 2007) has been a challenging problem to solve. Why do antitumour T cells fail to completely and reliably destroy tumours *in vivo* even when the ability to recognize tumours is not the limiting factor and there are large numbers of antitumour T cells present (Harlin *et al*., 2006) or when very high numbers of highly lytic antitumour T cells are injected in a cancer patient (Marincola *et al*., 2000; Rosenberg, 2001; Harlin *et al*., 2006) or tumour-bearing mice (Hanson *et al*., 2000)? What is present in the tumour microenvironment *in vivo* that prevents tumour destruction by the tumour-specific and highly lytic antitumour CD8 + T cells?

The starting answer appears to be that it is the accumulation of the intracellular immunosuppressor molecule, cyclic AMP, which inhibits the antitumour T cells (Takayama *et al*., 1987, 1988b; Takayama and Sitkovsky, 1988a; Sugiyama *et al*., 1992). This mechanism of tumour protection was thought to be an unintended application of the otherwise life-saving anti-inflammatory mechanism that functions to protect normal tissues from overactive immune cells during antipathogenic immune response. The studies of immunosuppressive effects of intracellular cAMP were followed by focusing on the extracellular adenosine and A2AR, as the most likely candidates that trigger the accumulation of intracellular cAMP and thereby function in physiological immunosuppressive mechanisms (reviewed by Sitkovsky, 2003, 2004; Sitkovsky and Lukashev, 2005a; Sitkovsky and Ohta, 2005b).

As the naturally occurring Tregs also have high levels of intracellular cAMP and Treg-mediated suppressive activity can be abolished by either cAMP antagonists and/or by gap

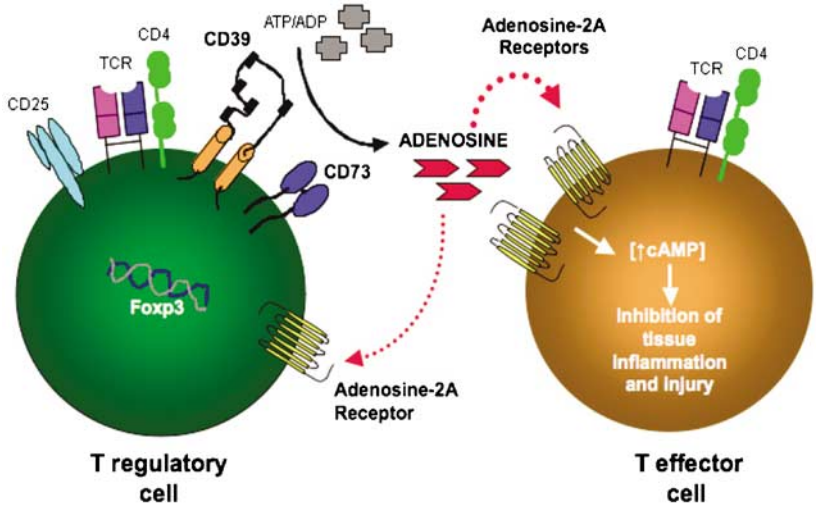

Figure 1 A2A adenosine receptor antagonist is expected to prevent inhibition of T cells by extracellular adenosine in inflamed or cancerous tissues. (**a**) The tumour tissue-produced extracellular adenosine inhibits incoming and TCR-activated antitumour T cells through activation of the cAMP-elevating A2A subtype of A2 adenosine receptors in the inflammatory or tumour microenvironment. The production of extracellular AMP adenosine is mediated by CD39 ectoenzyme, while the extracellular adenosine generation from extracellular AMP is mediated by CD73 ectoenzyme. (**b**) The T cells can be also inhibited by extracellular adenosine catalysed by CD39 and CD73 ectoenzymes expressed on the Treg. Treatment with A2A receptor antagonist may release antitumour T cells from inhibition in TME and facilitate the antitumour T-cell response. TCR, T-cell receptor.

junction inhibitors, it was recently proposed that Tregs may inhibit T cells by the intercellular transport of cAMP (Bopp *et al*., 2007).

cAMP-elevating Gs-protein-coupled A2A and A2B adenosine receptors and 'adenosinergic' ligands

Extracellular adenosine has been found to be the important and dominant inflammation-inhibiting molecule Sitkovsky (2003). The immediacy and ubiquity argument in the choice of potential inflammation-terminating signals is important, as this may ensure that the termination of excessive collateral inflammatory damage will be fast enough to protect still healthy tissues from the ongoing immune response. Our testing of adenosine was in agreement with earlier theoretical consideration of extracellular adenosine as a 'retaliatory' molecule (Newby, 1984). However, extracellular adenosine and cAMP-elevating A2AR have not been the only candidates. Other Gs-protein-coupled cAMP-elevating receptors including histamine, prostaglandins, β-adrenoceptors and their ligands also have been shown to be capable of immunosuppression, albeit if pharmacologically activated. Therefore, without the genetic *in vivo* evidence, we have already demonstrated that A2AR would have equivalent claims to efficacy as compared to other G-protein-coupled receptors shown to be as important physiological immunosuppressors (reviewed by Sitkovsky, 2003).

Intracellular sources and extracellular derivation of adenosine

Extracellular adenosine is shown to be produced in tumour microenvironment *in vivo* (Ohta *et al*., 2006) either due to adenosine kinase inhibition in hypoxia (Decking *et al*., 1997) and/or due to activities of ectonucleotidases ((Synnestvedt *et al*., 2002)) and/or due to overexpression of selective ATPase/ADPase on differentiated human melanoma cells (Dzhandzhugazyan *et al*., 1998) and other cells inclusive of the vasculature (Jackson, 2007).

The inhibition of intracellular adenosine kinase by hypoxia could also contribute in the extracellular adenosine accumulation. It has been shown that adenosine formation is proportional to the AMP substrate concentration and that hypoxia decreases adenosine kinase activity. The inhibition of adenosine kinase results in shunting intracellular adenosine from the salvage pathway to extracellular release. The 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 (Decking *et al*., 1997). Although not yet directly tested in hypoxic tumours and other tissues, this mechanism was suggested to be important in the high sensitivity of the cardiac adenosine system to impaired oxygenation.

In addition to intracellular sources of adenosine, the adenosine production through adenine nucleotide metabolism at the vascular and other cells surface may also trigger an endogenous anti-inflammatory response during hypoxia (Eltzschig *et al*., 2004). This is secondary to heightened activities of hypoxia-induced CD39, which converts ATP/ADP (converting ATP/ADP to AMP) and also of CD73 ecto-5′-nucleotidase (converts AMP to adenosine). This was supported in studies of CD39- and CD73-deficient animals with the CD39/ENTPD1 (Enjyoji *et al*., 1999) being recognized as the key cell-surface ectonucleotidase (Robson *et al*., 2005).

T regulatory cell generated immunosuppressive extracellular adenosine

FoxP3 + CD25 + CD4 + regulatory Treg ensure immunological self-tolerance by actively suppressing pathological and physiological immune responses (Sakaguchi, 2000; Shevach, 2006; Rudensky and Campbell, 2006). There are many clinical situations such as cancer immunotherapy or autoimmune diseases where it is important to manipulate (inhibit vs activate or adoptively transfer) Tregs to accomplish the desirable clinical outcome. It was previously shown in animal studies that the transfer of Tregs can be used to treat autoimmune diseases or to prevent transplant rejection (reviewed by Bluestone *et al*., 2007) by a cell-based tolerogenic therapy. Inhibition of the deleterious tumour infiltrating Tregs is considered highly desirable to improve the immunotherapy of cancer (reviewed by Shevach, 2004; Frumento *et al*., 2006; Quezada *et al*., 2006; Danese and Rutella, 2007; Wang and Wang, 2007).

The understanding of the mechanisms of immunosuppressive effects of Tregs is important not only to plan correct drug interventions in treatment of many diseases but also to develop better methods of cell culture to generate sufficient numbers of Tregs *in vitro* to be used later in immunotherapy *in vivo* (Fantini *et al*., 2007).

Tregs contribute to the production of immunosuppressive extracellular adenosine thereby recruiting the adenosinergic pathway to downregulate immune responses (Kobie *et al*., 2006; Deaglio *et al*., 2007) by inhibiting T cells and myeloid cells through A2AR. Kobie *et al*. (2006) have shown that CD73 (5′-ectonucleotidase) is expressed by CD25 + (FoxP3 +) Tregs that suppress T-cell proliferation but do not secrete interleukin-2 in standard Treg assays. They suggested that CD73 on Tregs converts extracellular 5′-AMP to adenosine, which then suppresses proliferation of activated T effector cells, as was shown to be the property of A2AR in earlier studies on A2AR-expressing murine and human T cells (Huang *et al*., 1997; Koshiba *et al*., 1997, 1999). A more complete picture have emerged when Deaglio *et al*. (2007) demonstrated that the coordinated coexpression of CD39 ecto-ATPase/ADPase and CD73 ecto-5′-nucleotidase provides specific surface markers of Tregs. Tregs thus have a unique biochemical signature amongst T cells in that they generate adenosine pericellularly. Treg function required the coordinated expression of the A2AR on activated T effector cells to enable the specifically mediated immunosuppression (Figure 1).

Extracellular A2A and A2B adenosine receptors on immune cells

There are four different and widely distributed adenosine receptors: A1, A2A, A2B and A3 (Fredholm *et al*., 1999, 2001;

Linden, 2001). The high-affinity A1 receptor and low-affinity A3 receptor are Gi-protein-coupled. The cAMP-elevating Gs-protein-coupled A2 receptors are subdivided into high-affinity A2AR and low-affinity A2BR. Adenosine receptors are known to be immunosuppressive (reviewed by Linden, 2001; Thiel *et al*., 2003; Sitkovsky, 2003, 2004).

Recent important reports indicate that the immunosuppressive A2AR-mediated 'adenosinergic' mechanism that was shown to be critically important in the regulation of immune response *in vivo* due to A2AR-triggered and intracellular cAMP-mediated inhibition of T-cell receptor-activated T cells and of Toll-like-receptor-activated myeloid cells (Ohta and Sitkovsky, 2001; Lukashev *et al*., 2004; Thiel *et al*., 2005; Ohta *et al*., 2006) may also be employed by Tregs (Kobie *et al*., 2006; Deaglio *et al*., 2007) and the adenosinergic immunosuppression may account for the large part of effects of FoxP3 + Tregs (Deaglio *et al*., 2007). Therefore, Tregs should be now added to the short list of sources of extracellular adenosine in inflamed and cancerous tissues.

The CD8 + T cells, including antitumour CD8 + T cells and human T cells, predominantly express A2AR and A2BR and not the A3 receptor (Huang *et al*., 1997; Koshiba *et al*., 1997, 1999; Chambers *et al*., 2001; Fredholm *et al*., 2001; Sitkovsky, 2003, 2004). The cAMP-elevating signalling through A2AR or A2BR in T cells results in inhibition of T-cell receptor-triggered activation of T cells (Takayama and Sitkovsky, 1988a; Sugiyama *et al*., 1992; Koshiba *et al*., 1999; Torgersen *et al*., 2002) and of many effector functions, including proliferation, expansion and secretion by T cells of such important antitumour cytokines as interferon γ (Koshiba *et al*., 1997; Sun *et al*., 2000; Torgersen *et al*., 2002) and tumour-necrosis factor α (Poehlein *et al*., 2003).

Delayed negative feedback inhibition of immune cells by extracellular adenosine

In the course of studies that eventually led to the demonstration of the critical physiological role of extracellular adenosine in the regulation of immune cells *in vivo* (Ohta and Sitkovsky, 2001), we have provided both pharmacological and genetic *in vivo* evidence that:

(1) Intracellular cAMP is indeed the high-fidelity immunosuppressor that inhibits T-cell receptor signalling at early and late stages of T-cell receptor-triggered T-cell-activating pathway (Trenn *et al*., 1987; Sitkovsky *et al*., 1988; Takayama *et al*., 1988b; Takayama and Sitkovsky, 1989; Sugiyama *et al*., 1992). This mechanism ensures powerful inhibition of virtually all effector functions of T lymphocytes and makes intracellular cAMP a plausible candidate for being responsible for inhibition of T cells near or within tumours. These studies of cAMP justified subsequent considerations of cAMP-elevating G-protein-coupled receptors and search for a physiological ligand and specific G-protein-coupled receptor that downregulates inflammation *in vivo*; Activated T cells do express cAMP-elevating A2A and A2B, but not the A1 or A3 receptors (Huang *et al*., 1997; Ohta and Sitkovsky, 2001). However, it remains to be tested in careful studies of human T cells from normal volunteers and patients whether there are normal or pathological situations when human T cells may express A1 or A3 receptors;- Studies of A2AR expression in wild-type vs heterozygous vs A2AR gene-deficient mice established that there is gene-dose effect and no A2AR reserve—no spare receptors—on T cells (Armstrong *et al*., 2001). This lack of A2AR reserve has important consequences, indicating that it is the number of A2AR per T cell that is the limiting factor in determining the susceptibility to inhibition by adenosine in tissue microenvironments (Sitkovsky, 2004); this, in turn, suggests that individuals with excessive number of A2AR per T cell may have weaker immune response to pathogens, but they also may be less likely to have autoimmunity.

(2) Finally, it was demonstrated that the genetic removal of A2AR resulted in dramatic enhancement of inflammation in *in vivo* models of T-cell-mediated autoimmune and viral hepatitis and myeloid cell-mediated tissue damage in models of sepsis, liver and lung injury (Ohta and Sitkovsky, 2001; Lukashev *et al*., 2004; Thiel *et al*., 2005; Ohta *et al*., 2006).

Our data are in further agreement with the view that healthy normal tissues in acutely inflamed and hypoxic areas that remain under immune attack are protected from the continuing collateral immune damage by immunosuppressive signalling through extracellular A2 receptor on the surface of immune cells. This mechanism of normal tissue protection from overactive immune cells in inflamed areas may be triggered by excessive collateral immune damage to endothelial cells and microcirculation with ensuing interruption of normal blood and oxygen supply (Sitkovsky, 2004). In turn, it results in local tissue hypoxia (Sitkovsky, 2003, 2004).

The decrease in local tissue oxygen tension, hypoxia, is associated with accumulation of intracellular and extracellular adenosine and sufficiently high levels of extracellular adenosine trigger signalling by A2AR and/or A2BR on the surface of surrounding cells, including activated T and myeloid cells. This chain of events culminates in the inhibition of overactive immune cells in a delayed, negative-feedback manner due to the well-established immunosuppression by A2 adenosine receptor (Ohta and Sitkovsky, 2001; Thiel *et al*., 2003; Sitkovsky, 2003, 2004).

Taken together, our *in vitro* and *in vivo* observations indicate that A2AR and A2BR are powerful negative regulators of pro-inflammatory and antitumour activities of activated T cells. Although A2BR have been earlier implicated in the regulation of inflammation *in vivo* using A2AR and A2BR antagonist (Ohta and Sitkovsky, 2001; Thiel *et al*., 2005), the specific anti-inflammatory role has been subsequently confirmed using A2BR-deficient mice (Yang *et al*., 2006).

Novel pharmacological use of A2AR antagonists: cancer immunotherapy

The advance in understanding tumour biology and effectiveness of cancer immunotherapy may come from the

demonstration that the elimination of A2AR genetically or the inhibition of A2AR signalling using A2AR antagonists prevents inhibition of antitumour T cells and improves tumour rejection (Ohta *et al*., 2006).

A2AR functions as a nonredundant negative regulator of activated T cells to protect normal tissues from excessive collateral inflammatory damage. It has been proposed that A2AR may also 'misguidedly' protect cancerous tissues (Ohta *et al*., 2006). It was reasoned that if this were indeed the case, then the genetic inactivation or pharmacological antagonism of A2AR would prevent the inhibition of antitumour T cells and thereby improve tumour rejection by these 'de-inhibited' T cells.

Drugs that inhibit A2AR-mediated adenosinergic pathway may enhance antitumour immunity by preventing effects of extracellular adenosine produced from both tissue and Tregs. These important findings of increased activities of CD39 and CD73 ectoenzymes on Tregs that produce extracellular adenosine have important pharmacological implications, as the large part of immunosuppressive activities of Tregs may be prevented by the same extracellular adenosine-degrading, -metabolizing or -antagonizing drugs that have been proposed to prevent the adenosinergic immunosuppression (Ohta *et al*., 2006).

Many solid tumours are characterized by an insufficient oxygen supply and transient or chronic hypoxia in some microenvironments (Vaupel *et al*., 2001; Harris, 2002). Tumour hypoxia may contribute to the propagation of oncogenic signals in the tumour microenvironment (Laderoute *et al*., 2000) and tumour hypoxia is associated with poor prognosis (Evans and Koch, 2003; Giatromanolaki *et al*., 2003). The progress has been made in measuring and discriminating effects of moderate vs deep tumour hypoxia (Evans and Koch, 2003; Giatromanolaki *et al*., 2003), and new conceptual and methodological approaches in this area of cancer research offer novel opportunities for other studies, far beyond cancer research.

Indeed, an interrupted blood supply and transient or chronic hypoxia in some microenvironments are observed not only in cancerous tissues (Vaupel *et al*., 2001; Harris, 2002; Evans and Koch, 2003; Giatromanolaki *et al*., 2003; Giatromanolaki *et al*., 2003), but also in inflamed normal tissues (reviewed by Ohta and Sitkovsky, 2001; Lahat *et al*., 2003; Sitkovsky, 2003, 2004).

The hypoxia-associated accumulation of intracellular adenosine in tumours and subsequent transport or diffusion of adenosine from the cell into extracellular space may contribute to extracellular adenosine accumulation in tumour microenvironment (Wang *et al*., 1994; Bodin and Burnstock, 1995; Decking *et al*., 1997; Kobayashi *et al*., 2000; Eltzschig *et al*., 2004; Ohta *et al*., 2006).

Careful estimation of levels of extracellular adenosine near and within studied tumours using the equilibrium microdialysis probe and HPLC has provided evidence for extracellular adenosine accumulation in hypoxic tumour tissue microenvironments. However, no definitive measurements of extracellular adenosine concentration in tissues *in vivo* can be obtained with existing analytic technologies (Ohta *et al*., 2006). Other authors, however, did provide their estimation as about 0.5 μM (Blay *et al*., 1997).

The prediction that there will be less inhibition of antitumour T cells in tumour microenvironment and therefore better rejection of tumours was tested using mice with genetic deletion of A2AR or using wild-type mice treated with A2AR antagonist. Experiments with different transplantable tumours demonstrated that genetic deletion of A2AR resulted in rejection of established immunogenic tumours by endogenously developed antitumour CD8+ T cells in approximately 60% of A2AR-deficient mice. No rejection was observed in control wild-type mice using the same number of inoculated tumour cells.

Of pharmacological interest is that the tumour growth retardation was also observed when mice with established tumours were given A2 adenosine receptor antagonists or when antitumour CD8+Tcells have been pretreated with short interfering RNA to lower the expression of A2AR and A2BR. Pharmacological treatment of mice with A2AR antagonists improved the antitumour T-cell-mediated inhibition of tumour growth, destruction of metastases and inhibited the neovascularization of cancerous tissues.

The improved tumour rejection after adoptive transfer of short interfering RNA-pretreated antitumour T cells suggests that effects of A2AR are T-cell autonomous. The inhibition of antitumour T cells through their A2AR in the adenosine-rich tumour microenvironment may at least partially explain the paradoxical coexistence of tumours and antitumour immune cells in some cancer patients (the 'Hellstrom paradox' as discussed above). It is appealing to propose to target the extracellular adenosine A2AR pathway as a cancer immunotherapy strategy to prevent the inhibition of antitumour T cells in the tumour microenvironment. The same strategy may prevent the premature termination of immune response and improve the vaccine-induced development of antitumour and antiviral T cells.

As melanoma tumour-rejecting A2AR-deficient mice had also developed autoimmunity (Ohta *et al*., 2006), it suggests that A2AR in T cells are also important in preventing autoimmune damage and that although using A2AR pathway inhibitors may improve antitumour immunity, the recruitment of this pathway by selective drugs is expected to attenuate the autoimmune tissue damage. Therefore, the design of A2AR antagonist-combined cancer immunotherapy should take into consideration the benefits of more effective tumour elimination vs need to monitor patient for signs of autoimmunity.

In addition, the vascular expression of CD39 and coordinated generation of adenosine also impact tumour growth in mutant mice. Deletion of Cd39/*Entpd1* inhibits angiogenesis in Matrigel (Goepfert *et al*. Circulation 2001) and also causes decreased growth of implanted melanoma tumours. Experimental data indicate clear links within CD39/ENTPD1, extracellular nucleotide/adenosine-mediated signalling and vascular endothelial cell integrin function that, at least in part, impact upon angiogenesis and tumour growth (Jackson, 2007).

Preliminary studies also suggest that CD39 expression on mutant adoptively transferred tumour infiltrating T cells in turn markedly inhibits and hence impacts tumour growth despite adequate angiogeneic responses from wild-type vasculature.

Conclusions and future directions

Taken together, our *in vitro* and *in vivo* observations show A2AR and A2BR to be powerful negative regulators of pro-inflammatory and antitumour activities of activated T cells. Although A2BR have been earlier implicated in the regulation of inflammation *in vivo* using A2AR and A2BR antagonist (Ohta and Sitkovsky, 2001; Thiel *et al*., 2005), their anti-inflammatory role was subsequently confirmed using A2BR-deficient mice (Yang *et al*., 2006). Ectonucleotidases upon Tregs and dendritic cells appear to metabolize extracellular nucleotides to generate adenosine to specifically modulate T effector and other immune cells in this manner. Hence, Cd39/Entpd1-null mice exhibit major immunological phenotypes in keeping with the loss of adenosinergic mechanisms (Deaglio *et al*., 2007).

Our studies also point to promising novel indications of A2AR antagonists or inhibition of CD39 in cancer immunotherapy and in blocking angiogenesis (Figure 1). Comparable effects are expected with infectious disease vaccines. Observations of autoimmunity during melanoma rejection in A2AR-deficient mice suggest that A2AR in T cells is also important in preventing autoimmunity. Hence, this approach using A2AR antagonists requires caution in patients with ongoing acute inflammation. It is also appealing to extend insights gained in studies of negative regulators of immune response in models of autoimmune and viral hepatitis, acute inflammation, and antitumour CD8+ and CD4+ T-cell-mediated cancer immunotherapy (Ohta and Sitkovsky, 2001; Thiel *et al*., 2005; Ohta *et al*., 2006) to novel strategies to improve treatment of infectious diseases. Similarly, the emerging threats of drug-resistant bacteria require the development of novel therapeutic strategies to sufficiently enhance the immune response of the host and destroy bacteria even in the absence of effective antibiotics.

Future development of anti-adenosinergic drugs or regulators of ectonucleotidases with rational management of diseases requires better understanding of mechanisms of tissue damage and organ failure. The drug development should be based on extensive basic and applied research to understand mechanisms that trigger or downregulate the immune response and thereby ensure the fine balance between desirable destruction of the pathogen and undesirable collateral damage to 'innocent' bystanders in normal tissues of vital organs (Sitkovsky, 2004; Ohta *et al*., 2006).

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Conflict of interest

The authors state no conflict of interest.

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