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Selective Inhibition of KCa3.1 Channels Mediates Adenosine Regulation of the Motility of Human T Cells

Ameet A. Chimote,* Peter Hajdu,* Vladimir Kucher,* Nina Boiko,* Zerrin Kuras,* Orsolya Szilagyi,* Yeo-Heung Yun,† and Laura Conforti*

Adenosine, a purine nucleoside, is present at high concentrations in tumors, where it contributes to the failure of immune cells to eliminate cancer cells. The mechanisms responsible for the immunosuppressive properties of adenosine are not fully understood. We tested the hypothesis that adenosine’s immunosuppressive functions in human T lymphocytes are in part mediated via modulation of ion channels. The activity of T lymphocytes relies on ion channels. KCa3.1 and Kv1.3 channels control cytokine release and, together with TRPM7, regulate T cell motility. Adenosine selectively inhibited KCa3.1, but not Kv1.3 and TRPM7, in activated human T cells. This effect of adenosine was mainly mediated by A2A receptors, as KCa3.1 inhibition was reversed by SCH58261 (selective A2A receptor antagonist), but not by MRS1754 (A2B receptor antagonist), and it was mimicked by the A2A receptor agonist CGS21680. Furthermore, it was mediated by the cAMP/protein kinase A isoform (PKAI) signaling pathway, as adenylyl-cyclase and PKAI inhibition prevented adenosine effect on KCa3.1. The functional implication of the effect of adenosine on KCa3.1 was determined by measuring T cell motility on ICAM-1 surfaces. Adenosine and CGS21680 inhibited T cell migration. Comparable effects were obtained by KCa3.1 blockade with TRAM-34. Furthermore, the effect of adenosine on cell migration was abolished by pre-exposure to TRAM-34. Additionally, adenosine suppresses IL-2 secretion via KCa3.1 inhibition. Our data indicate that adenosine inhibits KCa3.1 in human T cells via A2A receptor and PKAI, thereby resulting in decreased T cell motility and cytokine release. This mechanism is likely to contribute to decreased immune surveillance in solid tumors. The Journal of Immunology, 2013, 191: 000–000.

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denosine is an anti-inflammatory purine nucleoside that is released by cells in response to stress and hypoxia (1). Although adenosine provides a protective mechanism that controls inflammation, it becomes an immunological problem in tumors (2, 3). Indeed, accumulation of adenosine is characteristic of the hypoxic microenvironment of solid tumors, where it can reach levels 100-fold those of normal s.c. tissues (4). Importantly, because of its known immune-suppressive properties, adenosine has thus been associated with tumor progression, enhanced metastatic potential, and poor prognosis.

Adenosine is synthesized extracellularly by the phosphohydrolysis of ATP and ADP to AMP through the enzyme ecto-5′ nucleotidase (CD73) (2). Accumulation of adenosine in the tumor microenvironment is facilitated by hypoxia and by regulatory T cells (Treg) (2, 5). Hypoxia increases CD73 expression and reduces the expression adenosine degrading enzymes (2, 6). Treg, which are distinguished from other T cell subsets by the dual expression of CD39 and CD73, produce adenosine and use it to suppress effector T cells (7, 8). Adenosine signaling is mediated by four subtypes of G-protein–coupled receptors: A1, A2A, A2B, and A3. Of these, the A2A receptor is the predominant subtype in T lymphocytes and plays an important role in mediating adenosine’s immune suppressive effects: its stimulation impairs T cell activation and effector functions such as proliferation and secretion of cytokines like IFN-γ and IL-2 (9–14). In the tumor setting in particular, it has been shown that activation of A2A receptors results in decreased immune surveillance and tumor survival (15). Consequently, A2A receptor knockdown leads to T cell–dependent tumor rejection (15). Another important activity of adenosine, which further contributes to its immune-suppressive properties, is its ability to inhibit T cell trafficking (16). Recent findings in human lung mast cells suggested that the effect of adenosine and A2A receptor stimulation on cell migration may involve KCa3.1, a Ca2+-activated K+ channel also expressed in lymphocytes (17). Although many aspects of adenosine regulation of T cell function are now understood, the effects of adenosine on ion channels expressed in the T lymphocytes and their functional consequences are not known.

Ion channels are important regulators of T cell effector functions (i.e., cytokine release and proliferation) and motility (18). The main ion channels expressed in T cells are two K+ channels (the voltage-dependent Kv1.3 and the calcium-activated KCa3.1), the Ca2+-release activated Ca2+-channel and the Mg2+-permeant transient receptor potential melastatin 7 (TRPM7) channel. These channels act in concert to finely tune membrane potential and Ca2+ signaling (19, 20). Unequivocal evidence exists of the importance of ion channels in T cell activation as blockade
of these channels inhibit cytokine production/release (19, 20). The importance of ion channels in T cell motility is less understood, whereas, in other cell types, ion channels have been shown to regulate various aspects of cell migration including cell volume and F-actin polymerization/depolymerization necessary for the propulsion of the cell (18, 21, 22). We have recently shown that KCa3.1 and TRP75 regulate the motility of activated human T cells (23).

The current study was undertaken to study the effects of adenosine on ion channels in T lymphocytes and their downstream functional outcomes. We present evidence in this study that adenosine inhibits KCa3.1 channels in activated human T cells, thereby decreasing T cell motility and cytokine release. This mechanism is likely to contribute to decreased immune surveillance in hypoxic and adenosine-rich tumors.

Materials and Methods

Cells

CD3+ T cells were isolated from venous blood by E-rosetting (StemCell Technologies, Vancouver, BC, Canada) followed by Ficol-Paque density-gradient centrifugation (Nycodenz, Aurora, OH) or affinity purification as previously described (24). T cells were maintained in RPMI 1640 medium supplemented with 10% pooled human AB serum (Intergen, Milford, MA), 200 U/ml penicillin, 200 μg/ml streptomycin, and 10 mM HEPES (pH 7.4), supplemented with 2% FBS and 10% human AB serum. For the experiments described in this paper, cells were cultured in RPMI 1640 medium supplemented with 10% pooled male human AB serum (Intergen, Milford, MA), 200 U/ml penicillin, 200 μg/ml streptomycin, and 10 mM HEPES (pH 7.4). The cells were activated with either 4–10 μg/ml anti-human CD3 Abs, and 10 μg/ml anti-human CD28 Abs (BD Biosciences, San Jose, CA). Cells were resuspended (24). T cells were maintained in RPMI 1640 medium supplemented with 10% pooled human AB serum (Intergen, Milford, MA), 200 U/ml penicillin, 200 μg/ml streptomycin, and 10 mM HEPES (pH 7.4). The cells were activated with either 4–10 μg/ml anti-human CD3 Abs and 10 μg/ml anti-human CD28 Abs (BD Biosciences, San Jose, CA) for 72–96 h. Blood was obtained from healthy volunteers or blood bank donors (unused blood units) and maintained (in mM): 120 CH3O3S-Cs, 5 Cl−, 3.1 CaCl2, 10 BAPTA, and 10 glucose (pH 7.4). Cells were maintained in a cell culture incubator at 37˚C for 2 h before starting the migration experiments. After this time, coverslips were transferred to a microscopy chamber heated at 35˚C. Time-lapse microscopy was performed using the IncytIm3XMTD imaging system (Intracellular Imaging, Cincinnati, OH), and bright-field images were acquired at the rate of 20 images/min (23). The analysis and cell tracking was performed using Meta Morph software (Molecular Devices, Sunnyvale, CA). Polarized motile cells were considered for analysis using the criteria described earlier (23). Briefly, polarized T cells were defined as those displaying a leading edge and trailing uropod. Of these, the cells that were able to move away from their initial position with a minimum mean velocity of 1.5 μm/min were defined as migrating cells and included in our analysis. Cells that moved around a fixed contact point (i.e., did not travel any significant distance) or cells that remained at the same coordinate were excluded.

The same type of random amoeboid migration and comparable range of migration velocities were observed regardless of the type of ICAM-1 surfaces and medium used (23) (Supplemental Fig. 1).

Electrophysiology

Patch-clamp experiments were performed in activated T cells using Axopatch 200B amplifiers (Molecular Devices, Sunnyvale, CA) in whole-cell configuration as previously described (24). Currents were measured by -200 ms depolarizing voltage steps to +50 mV from an HP of 0 mV every 15 s (23).

Materials and Methods

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This blocker is highly selective for KCa3.1 with an IC50 of 25 nM to the specific KCa3.1 blocker TRAM-34 (23, 28) (Fig. 1A, 1B).

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Adenosine inhibits KCa3.1 channels via A2A receptors

Adenosine signaling is mediated by G-protein–coupled adenosine receptors. The A2A receptor is the predominant isoform expressed in human and mouse T cells, with A2B and A3 receptors expressed in lower abundance (9). Hence, we next determined whether A2A receptors mediate the inhibition of KCa3.1 channels induced by adenosine in activated human T cells. KCa3.1 channel activity was measured before and after exposure to adenosine, followed by SCH58261, a selective A2A receptor competitive antagonist, in the presence of adenosine (30). The KCa3.1 channel activity is reported as $G_{\text{KCa3.1}}$ normalized to maximum control conductance (before addition of the drug). As shown in Fig. 3A, treatment with adenosine reduced KCa3.1 activity by 50%, commensurate with our findings in Fig. 1. Addition of SCH58261 to the bath, after KCa3.1 steady-state inhibition by adenosine, returned the KCa3.1 activity to 90% of its baseline value. At this point, addition of TRAM-34 produced a complete block of KCa3.1. The bottom panel of Fig. 3A summarizes these results of $n = 12$ experiments in four donors. Contrary to the effect of A2A antagonist, the specific A2B antagonist MRS1754 did not reverse the effect of adenosine, indicating that the effect of adenosine on KCa3.1 channels is not mediated by the A2B receptor (Fig. 3B) (31). The role of A3 receptors in adenosine-induced inhibition of KCa3.1 was further confirmed by treating the cells with the A3 receptor agonist CGS21860: it induced a rapid and significant decrease in KCa3.1 conductance (Fig. 3C, top panel) (32). The degree of inhibition by CGS21860 was less as compared with that reported earlier, which can be attributed to the difference in expression system (native T cells versus overexpressed receptors).
in Chinese hamster ovary) and the readout method (binding assay versus patch-clamp technique) (Fig. 3C) (33).

In T cells, A\textsubscript{2A} signaling is mediated by cAMP and protein kinase A (PKA). The downstream effect of the A\textsubscript{2A} receptor is the activation of adenylyl cyclase and, consequently, cAMP production and PKA activation (12). cAMP, along with the PKA isoform type I (PKAI), negatively regulates cytokine production (34). Experiments were thus designed to determine whether an identical signaling pathway is involved in adenosine-induced inhibition of KC\textsubscript{a3.1}. The effect of adenosine on KC\textsubscript{a3.1} was achieved. (A, bottom panel) Blocked percentage of GK\textsubscript{a3.1} after treatment with 1 nM adenosine alone and adenosine plus 1 nM SCH58261 (n = 12, four donors). (B, top panel) Representative normalized GK\textsubscript{a3.1} are plotted before and after addition of 10 nM SCH58261 was applied to the bath with adenosine (n = 8, three donors). (B, bottom panel) Comparison of the percent inhibition of GK\textsubscript{a3.1} in cells treated with 1 nM adenosine alone and after addition of 50 nM A\textsubscript{2A} receptor antagonist MRS1754 in the presence of adenosine (n = 21, six donors) or 1 nM CGS21680 (n = 21, six donors).

FIGURE 3. Adenosine inhibits KC\textsubscript{a3.1} channels via A\textsubscript{2A} receptors. (A, top panel) Normalized GK\textsubscript{KCa3.1} (relative to the maximum conductance prior to the addition of adenosine) for a representative T cell upon perfusion with 1 μM adenosine alone and together with 1 μM SCH58261. TRAM-34 (10 μM) was added at the end of the experiments as positive control. SCH58261 was applied to the bath with adenosine still present in the bath, after the steady-state inhibition of KC\textsubscript{a3.1} by adenosine was achieved. (A, bottom panel) Blocked percentage of GK\textsubscript{KCa3.1} values is shown in T cells perfused with 1 μM adenosine alone (n = 14, three donors) or in presence of either the adenylyl cyclase inhibitor 2′,5′-dideoxyadenosine (100 μM; Fig. 4B) (36).

Overall, our results demonstrate that A\textsubscript{2A} receptors and cAMP/PKAI mediate the inhibitory effect of adenosine on KC\textsubscript{a3.1} channels in activated CD\textsuperscript{3} T cells. Still, the functional implications of KC\textsubscript{a3.1} modulation by adenosine are not understood. Recent reports have indicated that adenosine inhibits cell motility, but the mechanisms are poorly understood, although KC\textsubscript{a3.1} has been implicated in the effect of adenosine on the motility of human lung mast cells (17, 37). KC\textsubscript{a3.1} has been shown to play a vital role in the migration of activated T cells (23). Therefore, we investigated the effect of adenosine on T cell migration and the possible role of KC\textsubscript{a3.1}.

Adenosine inhibits T cell migration

The motility of T cells was studied using ICAM-1 surfaces without chemokine gradient. ICAM-1 interacts with the integrin LFA-1 on the T cell membrane and LFA-1 cross-linking can induce by itself T cell polarization and migration (22). Binding of ICAM-1 to LFA-1 results in LFA-1 activation and the downstream signaling cascade that leads to F-actin polymerization and forward motion (22). ICAM-1 surfaces are routinely used to study integrin-dependent migration. Integrins regulate the migration and retention of T cells during inflammation as they are involved in the extravasation of T cells into tissues and interactions with the extracellular matrix (22). To study the effect of adenosine on T cell migration, the motility of single T cells on ICAM-1 surfaces was recorded for 20 min before and after application of adenosine (Fig. 5A). T cells migrate on these surfaces by random amoeboid walk, as previously described (Supplemental Fig. 1A) (23). We observed that adenosine reduced the velocity of migrating T cells by 44% (Fig. 5A, 5B). The mean baseline velocity of 3.98 ± 0.26 μm/min decreased...
significantly to 2.22 ± 0.21 μm/min after addition of adenosine (n = 32, three donors; p < 0.01; Fig. 5B). The effect of adenosine was also mimicked by CGS21680. The mean baseline velocity of migrating T cells decreased by 54% in presence of CGS21680: from 5.23 ± 0.30 to 2.43 ± 0.24 μm/min (n = 26, three donors; p < 0.01; Fig. 5C).

Similar to adenosine, and in accordance with our earlier reported findings, the specific KCa3.1 blocker TRAM-34 inhibits T cell migration in activated CD3⁺ cells (Fig. 5D) (23). We observed a 43% decrease in the mean velocity, which decreased from 9.26 ± 0.35 to 5.32 ± 0.28 μm/min after addition of 25 nM TRAM-34 (n = 9, two donors; p < 0.01; Fig. 5D). This effect of TRAM-34 was produced by a concentration that, similarly to adenosine 1 μM, inhibits 50% of the KCa3.1 current. No further inhibition was produced by higher concentrations of TRAM-34 (500 nM TRAM-34 induced a 48% decrease in the mean velocity, which decreased from 4.01 ± 0.36 to 2.08 ± 0.18 μm/min after TRAM-34, n = 8, one donor; p < 0.01). In agreement with previous findings, the motility of migrating activated human T cells did not depend on Kv1.3, as treatment with the Kv1.3-specific blocker ShK did not change the mean velocity (p = 0.98; Fig. 5E) (23). These findings raise the possibility that adenosine effect on motility may be due to KCa3.1 inhibition. Still, we cannot exclude that the effect of adenosine on T cell motility may involve the other channel that regulates the motility of activated human T cells, TRPM7 (23). Because the effect of adenosine on TRPM7 is not known, we studied whether TRPM7 channel activity is influenced by adenosine.

Adenosine does not modulate TRPM7 currents

TRPM7 currents were measured in activated T cells before and after addition of adenosine. 2-Aminoethoxydiphenyl borate (2-APB), a nonspecific TRPM7 blocker, was used as positive control. The actual TRPM7 current recordings are illustrated in Fig. 6A. The effect of adenosine and 4-APB is reported as percentage of TRPM7 inhibition, measured at 100 mV. As shown in Fig. 6, there was no reduction in TRPM7 current when cells were perfused with 1 μM adenosine (n = 6), whereas the current was largely inhibited by the TRPM7 inhibitor 2-APB at 250 μM concentration (23). Thus, out of the two channels modulating the migration of activated T cells, only KCa3.1 is inhibited by adenosine. This further supports the possibility that the effect of adenosine on T cell motility is mediated by KCa3.1. Conclusive evidence could only be drawn if the effect of adenosine on T cell migration is abolished by KCa3.1 blockade.

The effect of adenosine on T cell migration is conveyed by KCa3.1

Experiments were performed to determine the effect of adenosine on T cell migration in presence of TRAM-34. The following experimental protocol was implemented: the migration of a single T cell was recorded for 6 min in regular medium (without adenosine and/or TRAM-34). After this time, TRAM-34 (500 nM, 20-fold higher than the IC₅₀ value) was added to the bath solution, and motility was measured for 6 more min (28). This interval is sufficient to obtain a steady-state inhibition of KCa3.1 currents and T cell motility (23). Cells were then exposed to adenosine in the presence of TRAM-34 and the motility was followed for 6 more min. The corresponding velocities in normal bath solution (no drugs), in the presence of TRAM-34 and TRAM-34 with 1 μM adenosine, are shown in Fig. 7. Overall, we observed a 40% decrease in the mean velocity of T cells from a baseline value of 7.47 ± 0.20 to 4.39 ± 0.14 μm/min after addition of TRAM-34 (n = 12, three donors; p < 0.01). Addition of adenosine to the bath solution in the presence of TRAM-34 did not further decrease the mean velocity of the cells (4.46 ± 0.14 μm/min; n = 12, three donors; p = 0.76 as compared with TRAM-34–treated group). In these experiments, adenosine alone produced a 40% inhibition of the velocity (from 6.10 ± 0.33 to 3.69 ± 0.22 μm/min [n = 6 cells from one donor; p < 0.01]). Similar effects were observed in T cells that were activated by TCR stimulation with CD3/CD28 Abs (Supplemental Fig. 2). Overall, these data show no additive or synergistic effect of adenosine on T cell motility in presence of

**FIGURE 5.** Adenosine inhibits T cell migration via A₂A receptors. (A) Representative time-lapse bright-field microscopy of an activated T cell migrating on an ICAM-1 surface before and after addition of adenosine (1 μM). The asterisk indicates the starting point; the arrow points at the migrating cell, and the continuous line the covered distance. Scale bars, 5 μm. (B and C) Effect of adenosine and A₂A receptor agonist CGS21680 on T cell migration (both 1 μM). T cell migration was measured in the same T cell before and after treatment with either 1 μM adenosine ([B] n = 32, three donors) or 1 μM CGS21680 ([C] n = 26, three donors). The results were obtained tracking individual cells for 20 min before and after drug application. (D and E) Responses to KCa3.1- and Kv1.3-specific blockers. The experiments were conducted as indicated in (B) and (C), using 25 nM TRAM-34 ([D] n = 9, two donors) or 10 nM ShK ([E] n = 6, one donor).
TRAM-34, indicating that KCa3.1 is the effector protein that mediates adenosine-induced inhibition of T cell migration.

Along with cell migration, KCa3.1 channels regulate cytokine release in T lymphocytes (29). Hence, we wanted to study whether KCa3.1 channel inhibition mediates the effect of adenosine on cytokine release in T cells.

Adenosine suppresses IL-2 release via KCa3.1 channel inhibition

Activated T cells were restimulated for 24 h with CD3/CD28 Abs in presence of 1 μM adenosine, 250 nM TRAM-34, or 250 nM TRAM-34 along with 1 μM adenosine. For these experiments, erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride (30 μM) was added together with adenosine to prevent adenosine degradation and ensure its long-term efficacy (16). Untreated cells were used as controls. ELISA was then performed to measure IL-2 levels. Adenosine and TRAM-34 reduced IL-2 levels by 31 ± 6% (n = 3 donors) and 32 ± 7% (n = 3 donors), respectively (Fig. 8). Moreover, no additive effect of adenosine on IL-2 levels in the presence of TRAM-34 was detected (33 ± 1% inhibition; n = 3 donors, Fig. 8). These data show that adenosine decreases IL-2 production in activated T cells and provide evidence of a role of KCa3.1 in mediating adenosine’s effect on cytokine release.

Discussion

Adenosine is an anti-inflammatory signaling molecule that has been implicated in the failure of immune surveillance in solid tumors (2). In this study, we show, for the first time to our knowledge, a role for ion channels in mediating adenosine-induced inhibition of T cell motility and IL-2 release. This finding adds ion channels to the signaling pathway that mediates the immune suppressive properties of adenosine downstream to the A2A receptor.

Adenosine has long been known to suppress the activation of T lymphocytes and secretion of proinflammatory cytokines (9). Moreover, adenosine has been implicated in suppressing lymphocytes trafficking in response to tissue injury or infections (9). The biochemistry of adenosine signaling that leads to inhibition of cytokine production and proliferation has been thoroughly investigated in T lymphocytes, and it involves the stimulation of A2A receptor and activation of cAMP/PKAI (9). The effect of adenosine on T cell trafficking is instead less understood. Furthermore, to our knowledge, no information is available on the effect of adenosine on ion channels in T lymphocytes. To our knowledge, this manuscript is the first report of such an effect. On the contrary, adenosine has been known to regulate the activity of ion channels in other cell types. Adenosine inhibits voltage-dependent Ca2+ channels in PC12 cells and the TWIK-related acid-sensitive K channel 1 in carotid body cells (38, 39). Furthermore, ATP-sensitive K+ channels and Cl- channels in epithelial cells are also modulated by adenosine (40). Our finding indicates that human T lymphocytes respond to acute exposure to adenosine with a suppression of KCa3.1 activity. The sensitivity to adenosine is specific to KCa3.1, as other channels expressed in T cells, Kv1.3 and TRPM7, are not modulated by adenosine. Furthermore, the classic adenosinergic pathway that mediates the transcriptional effects of adenosine (A2A receptor and cAMP/PKAI) in T cells also regulates KCa3.1. Modulation of KCa3.1 by cAMP/PKA has been reported.

FIGURE 6. Adenosine does not affect TRPM7 current. (A) Current traces of TRPM7 channels in a T cell in the absence or presence of 1 μM adenosine and 200 μM 2-APB were registered upon 200 ms-long voltage-ramp protocol ranging from -100 to +100 mV (HP was set 0 mV). (B) The inhibited percentage of TRPM7 current upon application of adenosine and 2-APB (see Results).

FIGURE 7. T cell migration by adenosine is regulated by KCa3.1 channels. Mean velocity of activated T cells upon application of TRAM-34 (500 nM) alone and in combination with 1 μM adenosine (n = 12, three donors). *p < 0.05.

FIGURE 8. Inhibition of KCa3.1 channels by adenosine reduces IL-2 secretion in T cells. CD3/CD28 Abs activated T cells were reactivated in the presence of 1 μM adenosine, 250 nM TRAM-34, or both 1 μM adenosine and 250 nM TRAM-34 for an additional 24 h, and IL-2 levels were measured in the supernatants by ELISA. The decreases in IL-2 levels are reported as percentage values normalized to the untreated controls. The data are the average of three distinct donors; each sample was measured in duplicate.
in other cell types with different outcomes. Increased cAMP levels have been shown to enhance KCa3.1 currents in human erythrocytes, Xenopus oocytes, and rat submandibular acinar cells by a mechanism that appears to be dependent on PKA (41–43). Others have reported a different outcome in oocytes, where they showed a potent inhibition of KCa3.1 by the catalytic subunit of PKA due to direct phosphorylation of the channel itself (44). In HEK293 cells, KCa3.1 was reported to be modulated via a cAMP-dependent protein kinase-independent phosphorylation (41). Similar to our finding, an inhibitory effect of adenosine and A2A receptor stimulation on KCa3.1 has been reported in human mast cells (17).

Ion channels are important regulators of T cell activation, effector functions, such as cytokine release and proliferation (19), and motility (23, 45, 46). We have recently shown that KCa3.1 and TRPM7, which localize at the uropod of migrating cells, control the motility of activated human T cells (i.e., inhibition of KCa3.1 and downregulation of TRPM7 inhibit T cell motility) (23). Toyama et al. (47) have also implicated KCa3.1 channels in T cell and macrophage migration/accumulation into atherosclerotic lesions in ApoE−/− mice in vivo. We have speculated that KCa3.1 and TRPM7 channels may work in concert to control the intracellular Ca2+ levels necessary for T cell forward motion (18). The role of ion channels in cell motility is well established in many cell types, in which it has been shown that they control the motility by regulating membrane potential, Ca2+ homeostasis and cell volume (18). Further studies are necessary to define the mechanism by which KCa3.1 and TRPM7 control T cell motility. Moreover, additional studies are necessary to establish whether KCa3.1 channels only control motility of selective T cell subsets. It has been reported that Kv1.3, the other K+ channel of T cells, plays a role in the migratory capacity of resting human T cells in vitro and rat effector memory T cells in vivo (45, 46). It has been reported that the expression of Kv1.3 and KCa3.1 channels differs in different T cell subsets and depends on the activation state: activated CCR7− effector memory T cells express a high number of Kv1.3 channels and activated CCR7+ naive and central memory T cells express high levels of KCa3.1 (19). The studies we have conducted are on a mixed population of T cells (preactivated by PHA or CD3/CD28 stimulation) with a high prevalence of T cells expressing high levels of KCa3.1 channels (48). Thus, we cannot exclude that our finding may not be extendable to all T cell subpopulations, but only apply to those cells for which the predominant K+ channel is KCa3.1. Indeed, resting human T cells, which display low KCa3.1 expression, did not polarize and migrate on ICAM-1 surfaces (data not shown) (48). Yet, quiescent T cells display also an LFA-1 with a low affinity for ICAM-1, which could explain/contribute to the lack of migration in our experimental setting (49). Furthermore, the K+ channel phenotype of different T cell subsets in disease other than autoimmunity has yet to be defined.

Although ion channels play such important role in T cell motility, very little is known about their regulation in T lymphocytes. In this study, we showed that adenosine and A2A receptor stimulation selectively inhibit KCa3.1 channels. Importantly, we showed that adenosine inhibits T cell migration, and this effect is prevented by KCa3.1 blockade. This finding indicates that adenosine effect on motility occurs via KCa3.1 inhibition. A role for KCa3.1 in mediating adenosine effect on cell motility was also suggested in human lung mast cells by correlating the effects of adenosine on cell motility to those produced by KCa3.1 blockade, but no conclusive evidence were reported (17). Along with T cell motility, KCa3.1 channels are also responsible for cytokine production (29). Similarly, adenosine and A2A receptor stimulation has been shown to inhibit cytokine release (2, 50). We present in this study evidence that support a role of KCa3.1 in mediating adenosine’s blockade of cytokine.

The ability of adenosine to inhibit T cell motility and cytokine release may be part of a protective mechanism in place to reduce inflammation. Unfortunately, the same effects may contribute to the decreased immune surveillance of solid tumors. Adenosine is highly concentrated in tumor sites, where it is produced by tumor cells, stromal cells, and Treg (3, 51). There is strong clinical evidence that immune surveillance in cancer patients correlates with the infiltration of immune cells into the tumor (3). Yet, the infiltration of tumors by effector T cells is limited, and CD3+ T cells in tumors display low motility (3, 52, 53). Although trafficking of immune cells into tumors is of such importance, the factors that limit the movement of the immune cells inside the tumors are poorly defined. The data we have presented in this study suggest a new mechanistic paradigm by which adenosine, produced in the tumor microenvironment, reduces KCa3.1 channel activity in effector T cells, thus limiting the infiltration of T lymphocytes into the tumor mass. This raises the importance of ion channels in T cells as contributing factors of the failure of the immune system caused by the tumor microenvironment. We have shown that hypoxia, which is also characteristic of the tumor microenvironment, inhibits Kv1.3 channels in T lymphocytes, thus reducing Ca2+ signaling and proliferation (25, 26, 54, 55). This finding combined with the effect of adenosine on KCa3.1 raise the possibility that hypoxia and adenosine in the tumor microenvironment provide a multidirectional attack on T cells via ion channels’ inhibition, which ultimately contributes to the weakened immune defenses at the tumor site. Furthermore, our finding raise the possibility that Treg-mediated immune suppression is conveyed, at least in part, by KCa3.1 channels. It is well established that Treg generate and use adenosine to suppress effector T cells by acting on the A2A receptors on these cells (7). Our results indicate that KCa3.1 channels, downstream to the A2A receptor, may link Treg function to the suppression of effector T cells.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


ADENOSINE INHIBITS HUMAN T CELL MOTILITY


