Adenosine A$_{2A}$ receptor activation reduces infarct size in the isolated, perfused mouse heart by inhibiting resident cardiac mast cell degranulation

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Rork TH, Wallace KL, Kennedy DP, Marshall MA, Lankford AR, Linden J. Adenosine A$_{2A}$ receptor activation reduces infarct size in the isolated, perfused mouse heart by inhibiting resident cardiac mast cell degranulation. Am J Physiol Heart Circ Physiol 295: H1825–H1833, 2008; doi:10.1152/ajpheart.495.2008.—Mast cells are found in the heart and contribute to reperfusion injury following myocardial ischemia. Since the activation of A$_{2A}$ adenosine receptors (A$_{2A}$ARs) inhibits reperfusion injury, we hypothesized that ATL146e (a selective A$_{2A}$AR agonist) might protect hearts in part by reducing cardiac mast cell degranulation. Hearts were isolated from five groups of congenic mice: A$_{2A}$AR$^{-/-}$ mice, A$_{2A}$AR$^{-/-}$ mice, mast cell-deficient (KitW$^sh$/W$^sh$) mice, and chimeric mice prepared by transplanting bone marrow from A$_{2A}$AR$^{-/-}$ or A$_{2A}$AR$^{+/+}$ mice to radiation-ablated A$_{2A}$AR$^{+/+}$ mice. Six weeks after bone marrow transplantation, cardiac mast cells were repopulated with >90% donor cells. In isolated, perfused hearts subjected to ischemia-reperfusion injury, ATL146e or CGS-21680 (100 nmol/l) decreased infarct size (IS; percent area at risk) from 38 ± 2% to 24 ± 2% and 22 ± 2% in ATL146e- and CGS-21680-treated hearts, respectively (P < 0.05) and significantly reduced mast cell degranulation, measured as tryptase release into reperfusion buffer. These changes were absent in A$_{2A}$AR$^{-/-}$ hearts and in hearts from chimeric mice with A$_{2A}$AR$^{-/-}$ bone marrow. Vehicle-treated KitW$^sh$/W$^sh$ mice had lower IS (11 ± 3%) than WT mice, and ATL146e had no significant protective effect (16 ± 3%). These data suggest that in vivo, buffer-perfused hearts, mast cell degranulation contributes to ischemia-reperfusion injury. In addition, our data suggest that A$_{2A}$AR activation is cardioprotective in the isolated heart, at least in part by attenuating resident mast cell degranulation.

Langendorff; tryptase; ATL146e; CGS-21680; bone marrow chimera

Mast cells contribute to immune responses with both sentinel and effector roles in host defense and inflammation. The activation of mast cells has been found to have protective or deleterious effects in response to tissue injury or infection (22, 29). This is due in part to tissue-specific heterogeneity of mast cell function (3, 48). In the heart, degranulation of resident cardiac mast cells mediates injurious effects during experimental ischemia-reperfusion (I/R) injury and myocardial infarction (MI). These deleterious effects are mediated by multiple mechanisms, including a local renin-angiotensin axis (18, 26, 41), histamine and prostanooid-induced ventricular arrhythmogenesis (36), and the initiation of a cytokine cascade resulting in increased ICAM-1 expression and neutrophil extravasation (13, 42). In addition, resident cardiac mast cells contribute to the ventricular hypertrophic response during chronic cardiac volume overload (6, 16, 32, 33). In the isolated heart, oxidative stress from I/R is sufficient to stimulate degranulation of resident cardiac mast cells (13). Mast cell stabilizers such as ketotifen and low-dose carvedilol attenuate I/R-induced myocardial injury (15).

Adenosine or adenosine analogs such as 5′-N-ethylcarboxamidoadenosine or N$^6$-(3-Iodobenzyl)-adenosine-5′-N-methyluronamide primarily use the A$_3$ adenosine receptor (AR) to stimulate degranulation of rat or murine mast cells, whereas the A$_2B$AR is the principle activator of primed canine or human mast cells (2, 9, 23, 40, 47, 52). In some, but not all, mast cells, A$_2$AR activation reduces degranulation and migration by inhibiting the Ca$^{2+}$-activated K$^+$ channel KCNQ3 (8, 17). The effect of A$_2$AR agonism on in vivo murine cardiac mast cells is unknown. In vivo, A$_2$AR activation during reperfusion following coronary artery occlusion reduces mouse heart reperfusion injury by preventing lymphocyte uptake and activation (50). Here, we demonstrate that A$_2$AR activation inhibits cardiac mast cell degranulation. A$_2$AR activation or mast cell depletion protects the buffer-perfused isolated mouse heart from reperfusion injury, suggesting that mast cell degranulation contributes to reperfusion injury.

Materials and Methods

Animals. Mouse experiments were approved by the University of Virginia Animal Care and Use Committee. A$_{2A}$AR$^{-/-}$ [wild type (WT)], KitW$^sh$/W$^sh$ (mast cell deficient), and ubiquitous green fluorescent protein (uGFP) mice congenic with C57BL/6J were purchased from Jackson Laboratory. A$_{2A}$AR$^{-/-}$ mice from Jiang-Fan Chen (Boston University) were moved onto a C57BL/6J background.

Isolated, perfused mouse hearts. Metabolic, cellular, and functional responses to I/R injury were assessed using the isolated, perfused mouse heart as previously described (20). Adult male mice (10–12 wk old, 20 ± 5 g) were anesthetized with pentobarbital sodium, and hearts were excised and placed in ice-cold heparinized perfusion buffer. Hearts were retrograde perfused via an aortic cannula with a modified Krebs-Henseleit buffer [containing (in mmol/l) 118 NaCl, 25 NaHCO$_3$, 4.7 KCl, 1.2 KH$_2$PO$_4$, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 11 glucose, and 0.6 EDTA equilibrated with 95% O$_2$-5% CO$_2$ to an end PO$_2$ of 550 ± 50 mmHg]. Coronary perfusate flow was continuously monitored by an in-line ultrasonic flow probe (Transonic Systems, Ithaca, NY). Following 10 min of equilibration, hearts were paced for an additional 10 min at 425 beats/min via an electrode at the base of the right ventricle.

Experimental protocol. After 20 min of equilibration, pacing was halted, and hearts were subject to 30 min of global ischemia followed by 10.220.33.1 on July 7, 2017 http://ajpheart.physiology.org/ Downloaded from

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by 30 min of perfusion as previously described (1). Vehicle, CGS-21680 (final concentration: 100 mM; Fig. 1), or ATLA146e (final concentration: 100 mM; Adenosine Therapeutics Group, Charlotte, VA; Fig. 1) was infused at a 1% coronary perfusate flow rate throughout the reperfusion interval. Samples of coronary perfusate effluent (500 ± 50 µl) were collected at baseline and at 1, 2, 5, 10, 15, 20, 25, and 30 min of perfusion for trypase analysis (Fig. 2). Following reperfusion, hearts were stained with 1.5% triphenyltetrazolium chloride in PBS at 37°C to determine infarct size (IS). IS was expressed as the percent area at risk (AAR), where the AAR was considered to be the entirety of the left ventricle (global myocardial ischemia).

Trypsate release. Coronary effluent samples were analyzed for trypase content enzymatically as described by Lavens et al. (21). Briefly, 50 µl of coronary effluent perfusate were incubated with 50 µl of 0.8 mmol/l α-N-benzoyl-l-arginine-p-nitroanilide (up to 72 h at 37°C), and the nitroaniline product was measured colorimetrically at 405 nm (Wallac 1420 VICTOR2 plate reader, Perkin-Elmer, Waltham, MA). All samples and standards were run in duplicate, and tryptase values were calculated as micrograms per minute per gram of tissue. Values are expressed as percent changes from the preischemic baseline.

Bone marrow transplantation. Irradiated (600 rad twice at two 4-h intervals) 6-wk-old WT mice served as recipients for bone marrow transplantation as previously described (7). Donor mice (WT, A<sub>2a</sub>AR<sup>−/−</sup>, or uGFP C57Bl/6 mice) were anesthetized with pento-barbital sodium and killed. Marrow from femur and tibia was harvested by flushing the marrow cavity with RPMI-1640 medium (Invitrogen) plus 10% FBS to obtain a suspension of ~5 × 10<sup>7</sup> nucleated cells. Subsequent to irradiation, 3 × 10<sup>6</sup> previously harvested bone marrow cells were injected into the jugular vein of each mouse. Transplanted mice were fed chow containing 5 mM sulfamethoxazole and 0.86 mM trimethoprim and allowed to recover for 6 wk before experimentation. This procedure has consistently produced turnover of >85% circulating lymphocytes and >95% circulating granulocytes (7).

Isolation of primary mast cells. To assess the extent of mast cell turnover in various tissues, mouse primary peritoneal mast cells (mPPMC) as well as mouse primary lung mast cells (mPLMC) and mouse primary heart mast cells (mPHMC) were derived from bone marrow chimeric (BMC) mice where either uGFP or WT bone marrow was reconstituted into radiation-ablated WT mice. mPPMCs were collected by peritoneal lavage with 10 ml PBS. For the isolation of mPLMCs, mice were anesthetized, and a complete thoracotomy was performed. The pulmonary vasculature was perfused with 10 ml of saline, and the right and left lung lobes were removed and mechanically dissociated. The dissociated tissue was diluted with 10 ml PBS and placed on ice (52).

Isolation of mPHMCs was performed as previously described (10). Ventricular tissue was manually dissociated and incubated in 160 U/ml collagenase type II, 100 U/ml hyaluronidase, 1 U/ml pronase E, and 304 U/ml DNase I in PBS at 37°C. Supernatants collected after three 15- to 30-min digestion intervals were filtered through 70-µm nylon mesh and subsequently washed with PBS. Pooled cells were combined and washed twice in PBS.

Primary mast cell cultures. mPPMCs, mPLMCs, and mPHMCs were cultured in DMEM containing 10 ng/ml stem cell factor and 10 ng/ml IL-3; culture media were changed every other day for 1 wk (25, 52). Nonadherent cells were collected and transferred to fresh media. Cells were passaged in the same manner for 6 wk, at which time the population of granular cells that had grown out was analyzed by flow cytometry.

Immunostaining for flow cytometry. After 6 wk in culture, mPPMCs, mPLMCs, and mPHMCs were washed twice with PBS (1% BSA) and resuspended at 10<sup>7</sup> cells/ml in staining buffer (1% BSA and 0.1% sodium azide in PBS). Aliquots were labeled with anti-mouse CD45 (Becton Dickinson) and anti-mouse CD117 and anti-mouse FcεRI (eBioscience). Control samples were labeled with isotype-matched control antibodies. PBS (1 ml) was added along with the Aqua Live/Dead Fixable Dead Cell Stain Kit (Molecular Probes, Eugene, OR) for an additional 30 min. Stained cells were washed, fixed (1% paraformaldehyde in PBS), and resuspended with FlowJo software (Tree Star, Ashland, OR). Cell gates were created based on fluorescence minus one staining and live CD45-positive cells were gated on for analysis. Mast cells found in the live cell CD45-positive gate were identified as CD117 positive and FcεRI positive.

Radioligand binding for AR subtype specificity. Radioligand binding to recombinant human and mouse AR subtypes was performed as described by Murphree et al. (31).

Statistics. For IS determination, all groups were compared using one-way ANOVA with a Student-Newman-Keuls multiple-comparison test. For trypase analysis, all groups were compared using two-way ANOVA with a Bonferroni post test. For all tests, significance was established at $P < 0.05$. 

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**Fig. 1.** Chemical formulas of ATL146e and CGS-21680. Mast cells were subsequently cultured from dissociated lung pieces.
RESULTS

Effects of ATL146e and CGS-21680 in $\alpha_2$AAR$^{+/+}$ and $\alpha_2$AAR$^{-/-}$ mice. ATL146e and CGS-21680 at 100 nmol/l final concentrations did not cause any changes in coronary conductance (as measured by coronary perfusion flow rate) at any recorded time period in any experimental group (data not shown). When administered during reperfusion, ATL146e and CGS-21680 significantly decreased IS in WT mice (Fig. 3) from 38 ± 2% ARR to 24 ± 2% and 22 ± 2% AAR in ATL146e- and CGS-21680-treated hearts, respectively ($P < 0.05$). This effect was absent in $\alpha_2$AAR$^{-/-}$ mice, as ISs between vehicle- and ATL146e-treated animals were similar (44 ± 4% AAR vs. 38 ± 3% AAR in vehicle- and ATL146e-treated hearts, respectively; Fig. 3).

Tryptase release was not significantly different at baseline between WT and $\alpha_2$AAR$^{-/-}$ groups (Fig. 4A). However, baseline tryptase release was decreased in hearts from both vehicle- and ATL146e-treated KitW-sh/W-sh groups compared with hearts from mice with functional mast cells. ATL146e or CGS-21680 treatment significantly decreased tryptase release during reperfusion in WT hearts compared with vehicle-treated controls (Fig. 4B). ATL146e treatment had no effect in $\alpha_2$AAR$^{-/-}$ mice, confirming that the effects of ATL146e are mediated through the $\alpha_2$AAR (Fig. 4C). Interestingly, the peak tryptase release in vehicle-treated groups was significantly lower in $\alpha_2$AAR$^{-/-}$ mice compared with WT mice, possibly due to chronic stimulation of mast cells lacking inhibitor ARs.

Mast cell turnover in BMC mice. We conducted bone marrow transplantation experiments to selectively delete the $\alpha_2$AAR in bone marrow-derived cells (including mast cells). Since the turnover rate of murine cardiac mast cells was unknown, we examined the efficiency of mast cell repopulation using mice ubiquitously expressing GFP (uGFP) as the source of donor marrow. After mast cells had been harvested from the heart and other tissues, they were expanded in culture, and the fraction of GFP-positive cells was determined by flow cytometry. Cytometry was also performed on identical cultures that were derived from mice where bone marrow from WT mice had been transplanted to ablated WT mice. As shown in Fig. 5A, CD45-positive, FcεRI-positive, and CD117-positive (triple positive) cells were identified as mast cells. In bone marrow transplanted mice that received WT bone marrow, nearly all mast cells were confirmed to be GFP negative (Fig. 5B–D). Conversely, in bone marrow transplanted mice that received uGFP bone marrow, the majority of mast cells derived from the heart and lung were GFP positive, whereas peritoneal-derived mast cells were less than half GFP positive.
positive. The percentage of GFP-positive cells was 93.5% in mPHMCs (Fig. 5E), greater than 99% in mPLMCs (Fig. 5F), and 44.1% in mPPMCs (Fig. 5G). Thus, the turnover of resident cardiac mast cells occurs within the 6-wk period given for bone marrow reconstitution, as cultured cells from hearts of uGFP BMC mice were nearly all GFP positive. In addition, these results confirm that bone marrow ablation by radiation is nearly 100% effective in ablating resident cardiac mast cells, as very few surviving cells were GFP negative.

Cardioprotection by ATL146e is mediated through bone marrow-derived cells. Following transplantation of A2AAR−/− donor bone marrow to radiation-ablated WT recipients [knockout (KO)/WT], the average IS in vehicle-treated groups was 33 ± 3%, and the average IS in ATL146e-treated groups was not changed (38 ± 3%; Fig. 6). In contrast, in hearts from WT/WT BMT mice, the IS reduction in response to ATL146e was reconstituted (40 ± 1% vs. 22 ± 3%, P < 0.05). The ineffectiveness of ATL146e in reducing IS in KO/WT BMT chimeric hearts indicates that the target of ATL146e is A2AARs on bone marrow-derived cells. The reconstitution of ATL146e-mediated protection in WT/WT hearts demonstrates that there is little or no effect of bone marrow transplantation per se on the efficacy of ATL146e to reduce IS.

Tryptase release during reperfusion following myocardial ischemia was similar in vehicle- and ATL146e-treated hearts from KO/WT mice (Fig. 7A). In WT/WT hearts, ATL146e treatment reduced tryptase release (Fig. 7B). In concordance with IS data, the lack of effect of ATL146e in KO/WT hearts indicates that selective deletion of the A2AAR on bone marrow cells rendered cardiac mast cells insensitive to A2AAR activation. Conversely, ATL146e-reduced tryptase release in WT/WT BMC mice.

ATL146e does not protect KitW-sh/W-sh mice. Hearts from KitW-sh/W-sh mice had significantly lower ISs than either vehicle- and ATL146e-treated WT hearts (Fig. 8A). In addition, ISs in KitW-sh/W-sh hearts were significantly lower than those in all other groups tested. ATL146e treatment had no effect to further reduce IS compared with vehicle treatment, as IS in vehicle- and ATL146e-treated groups was 16 ± 3% versus 11 ± 3%, respectively (not significant; Fig. 8A). Mast cell deficiency therefore appears to be protective in the isolated, perfused heart. The lack of additional benefit from ATL146e treatment suggests that mast cells are necessary for the protective effects of A2AAR activation. As expected, little or no tryptase release was detected in hearts from KitW-sh/W-sh mice, and ATL146e treatment had no significant effect (Fig. 8B).

DISCUSSION

This study shows that the A2AAR agonists ATL146e and CGS-21680 reduce ex vivo I/R injury in the isolated, asanguineous mouse heart. Upon reperfusion following ischemia, cardiac...
mast cells degranulated as assessed by measuring tryptase release into the coronary effluent perfusate. Hearts from WT but not A2AAR−/− mice had reduced tryptase release on reperfusion with ATL146e or CGS-21680, indicating that A2AAR activation can reduce mouse heart mast cell degranulation. Hearts from mast cell-deficient mice also had greatly reduced ISs compared with hearts with mast cells. These findings suggest that inhibition of cardiac mast cell degranulation contributes to the cardioprotective effects of A2AAR agonists in the isolated perfused mouse heart. To our knowledge, this is the first study to show IS reduction in isolated, perfused hearts with an A2AAR agonist given at the onset of reperfusion; Maddock et al. (27) showed that A2AAR agonism attenuated I/R-induced myocardial stunning but did not measure IS. Our data agree with those of Cargnoni et al. (5), although they concluded that the protective target for A2AAR agonism is the cardiac myocyte. CGS-21680 did not significantly protect hearts from reperfusion injury in some previous studies (38, 44, 51). However, other studies have shown a protective effect for this compound (5), and the role of A2AAR activation in cardioprotection has been controversial. In the present study, CGS-21680 or ATL146e were cardioprotective when added to the coronary perfusate at 100 nmol/l. Findings with A2AAR−/− mice clearly implicate A2AARs as mediators of cardioprotection. We conclude that A2AAR agonists can reduce reperfusion injury in the isolated heart, but possibly only within a certain range of injury.

A2AARs are activated by ATL146e and CGS-21680 but also by adenosine produced in ischemic tissues. Recently, Morrison et al. (30) described the importance of A2AARs in postconditioning, defined as cardioprotection caused by short periods of ischemia following a long ischemic episode. Their data showed that treatment with ZM-241385 (an A2AAR-specific antagonist) or A2AAR-specific deletion abrogated myocardial postconditioning and that postconditioned-mediated protection through A2AAR involves ERK1/2 and Akt phosphorylation. Although the role of ERK1/2 and Akt phosphorylation on resident cardiac mast cells is not known, it is possible that A2AAR-mediated postconditioning is facilitated in part by inhibition of mast cell degranulation by adenosine generated by short-duration ischemic postconditioning episodes. Parikh and Singh (37) have provided evidence that cardiac mast cells may play a role in ischemic preconditioning, and these results may extrapolated to postconditioning as well.

Although the role of interstitial mast cells in in vivo MI has been established by several investigators, very few studies have focused on resident cardiac mast cells in isolated hearts. Bhattacharya et al. (4) and Reil et al. (42) showed that isolated hearts from mast cell-deficient mice release less IL-6 and TNF-α into the coronary effluent when these hearts had been subjected to I/R. Our findings show that resident cardiac mast

![Fig. 6. ISs in vehicle- and ATL146e-treated A2AAR+/+ and A2AAR−/− BMC mice.](image)

![Fig. 7. Tryptase release in vehicle- and ATL146e-treated A2AAR+/+ and A2AAR−/− BMC mice.](image)
cells contribute to I/R-induced IS expansion in the isolated heart. Consistent with this, Jaggi et al. (15) showed that low-dose carvedilol or ketotifen (both mast cell stabilizers) reduced myocardial injury following I/R, and these compounds had no effect on hearts degranulated with compound 48/80 prior to I/R. Mast cell-mediated myocardial damage occurs despite the relative paucity of mast cells in the C57Bl/6 mouse heart, as reported by Gersch et al. (12). It is possible that the role of resident cardiac mast cells in I/R is amplified in the isolated, perfused heart, as the induced ischemia is global rather than regional. Thus, all mast cells present in the myocardium are subject to I/R stress, whereas regional MI would selectively activate only mast cells in the AAR in the coronary occlusion models that are generally used in vivo.

We recently showed that the protective effect of ATL146e in murine in vivo MI is dependent on its action on CD4-positive T cells, as CD4-positive T cell-specific A2AAR deletion abolishes the protective effect of this drug (49, 50). These in vivo results implicate lymphocytes as the cell type of primary importance in MI. However, T cells are absent in the buffer-perfused heart and therefore do not mediate the damage caused by I/R, nor are they the target of cardioprotection by A2AAR agonists in this setting. Both mast cells and lymphocytes may be important inflammatory targets in vivo, as it is plausible that degranulation of resident cardiac mast cells is upstream of the infiltration of CD4-positive T cells in the pathology of MI. Frangogiannis et al. (11) have shown that in response to I/R, cardiac mast cells degranulate and release preformed TNF-α, which subsequently induces IL-6 expression in infiltrating mononuclear cells. IL-6 in turn upregulates myocardial ICAM-1 expression, increasing susceptibility to neutrophil-induced adhesion, extravasation, and cytotoxicity (43). In addition, evidence that mast cells can enhance T cell activation (i.e., cytokine production) through TNF-α secretion has been provided by Nakae et al. (34, 35). Thus, resident cardiac mast cells may initiate an inflammatory cascade during in vivo MI and subsequently stimulate the accumulation and activation of CD4-positive T cells in the infarct zone. Additional studies to examine the effect of mast cell deficiency on MI are necessary to further elucidate this possible mechanism.

Independent of any effects on lymphocyte trafficking or activation, our data indicate that mast cell degranulation is injurious to the isolated myocardium following I/R in the absence of infiltrating inflammatory cells. Mast cells are known to release various preformed cytotoxic substances such as serotonin, IL-8, keratinocyte chemoattractant, RANTES, endothelin, bradykinin, urocortin, and substance P, among others (46). In addition, mast cell degranulation releases several preformed proteases such as arylsulfatases, chymase, trypsin, and metalloproteinases, which may be directly damaging to cardiac myocytes and which may be responsible for the observed dependence of increased IS on mast cell degranulation. Recently, Mackins et al. (26) showed evidence for the activation of a local renin-angiotensin axis during I/R in isolated hearts initiated by cardiac mast cell-derived renin. It is plausible that through the activation of angiotensin II and NADPH oxidase, this axis could damage the myocardium directly (in addition to causing rhythm disturbances, as previ-

Table 1. Receptor binding affinities as determined by radioligand binding for ATL146e and CGS-21680 in the human and mouse A1AR, A2AAR, A3AR, and A2BAR

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<th>Human A2AAR</th>
<th>Mouse A2AAR</th>
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<tr>
<td></td>
<td>Low-Affinity Binding Site</td>
<td>High-Affinity Binding Site</td>
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<tr>
<td>ATL146e</td>
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<td>0.68±0.1</td>
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<tr>
<td>CGS-21680</td>
<td>197±27.3</td>
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Values are means ± SE of receptor binding affinity (K_i) values (in nmol/l unless otherwise noted). AR, adenosine receptor; NA, not available at the time of publication.
ously reported). Further studies are necessary to determine both the identity and effects of mast cell mediators on the heart. Mast cell mediators may influence in vivo MI by 1) directly damaging the myocardium, thus amplifying the cellular response to injury; 2) increasing the expression of adhesion molecules on vascular endothelial cells, thus increasing the recruitment of inflammatory cells; and 3) stimulating other proximal mast cells to degranulate and further exacerbate the inflammatory cytokine cascade.

Evidence suggesting the release of tryptase from isolated, perfused hearts has been previously reported by Matsui et al. (28), where tryptase-like protease activity correlated well with histamine release during ischemia. These investigators reported that the majority of mast cell degranulation occurs during ischemia with insignificant degranulation occurring during reperfusion. Importantly, this study was carried out by perfusing the heart with an ischemic buffer rather than by using no-flow global ischemia, as was done in the present study. In the present study, we clearly showed that most tryptase release occurs upon reperfusion and that mast cell degranulation contributes to IS expansion.

The role of adenosine in the modulation of mast cell responses has been extensively investigated, but controversy still exists regarding its precise role (24). Our data show that the administration of an A2AAR-selective agonist decreases I/R-induced degranulation in resident cardiac mast cells and that this inhibition is A2AAR dependent. These data are supported by other studies demonstrating the inhibitory capacity of the A2AAR on mast cell degranulation and are consistent with a general mechanism where Gi activation increases intracellular cAMP and suppresses mast cell degranulation (8, 17, 45). This is in contrast to the A3AR (Gi coupled), which promotes mast cell degranulation by increasing the intracellular Ca\(^{2+}\) concentration (17). Interestingly, other studies that have employed A3AR-specific agonists at the onset of reperfusion have not found an exacerbation of IS, although many of the agonists used also activate A2AARs (31). Indeed, as noted by the selectivity of ATL146e and CGS-21680 in the mouse (Table 1), we did not observe an increase in tryptase release from A2AAR\(^{−/−}\) hearts treated with ATL146e.

Mas cell cells are relatively long lived after they reach terminal differentiation in peripheral tissues. Our data indicate that after irradiation, both lung and heart mast cells are replaced by bone marrow-derived cells within 6 wk. In contrast, peritoneal mast cells did not turn over this quickly, as evidenced by the fact that 6 wk after GFP bone marrow transplantation, only ~44% of mast cells were GFP positive. The actual turnover rate of mast cells independent of irradiation is not known; however, some studies have suggested that these cells can reside in tissue for extended periods of time (19). Regardless, our data advocate the use of bone marrow transplantation as an effective method to examine mast cell-dependent tissue effects in both the lung and heart.

Interestingly, ISs in A2AAR\(^{−/−}\) hearts were not greater than ISs in WT hearts in the vehicle-treated group. The lack of IS exacerbation suggests that there is no endogenous, nonpharmacological protective value of A2AAR activation in the inhibition of mast cell degranulation during reperfusion. This may indicate that the endogenous adenosine that accumulates during ischemia is rapidly depleted during reperfusion, and A2AAR adenosinergic tone must be maintained during reperfusion to effectively inhibit mast cell degranulation.

An important limitation of the present study lies in the relative youth and health of the animals used. It is unknown what the role of resident cardiac mast cells is in aged animals with comorbid conditions (e.g., diabetes, hypercholesteremia, hypertension, etc.), a population that would more closely resemble the human population most at risk for MI. Several investigators have noted the role of mast cells in plaque formation and rupture during atherosclerosis (22); however, no data exist regarding the role of preplaque myocardial mast cells in these models. It is possible that other factors in these diseases influence the role of mast cells.

In summary, our data show that the administration of an A2AAR-selective agonist during reperfusion is cardioprotective in the isolated, perfused mouse heart via inhibition of resident cardiac mast cell degranulation. While mast cell mediators can directly damage the isolated heart, an important additional role of these mediators may be as chemotactic stimuli for other damaging leukocytes in vivo.

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DISCLOSURES

J. Linden is a paid consultant to the Adenosine Therapeutics Group.

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