Myocardial Infarct–Sparing Effect of Adenosine A2A Receptor Activation Is due to Its Action on CD4+ T Lymphocytes

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**Background**—We previously used adenosine A2A receptor (A2AR) knockout (KO) mice and bone marrow transplantation to show that the infarct-sparing effect of A2AR activation at reperfusion is primarily due to effects on bone marrow–derived cells. In this study we show that CD4+ but not CD8+ T lymphocytes contribute to myocardial ischemia/reperfusion injury.

**Method and Results**—After a 45-minute occlusion of the left anterior descending coronary artery and reperfusion, T cells accumulate in the infarct zone within 2 minutes. Addition of 10 μg/kg of the A2AR agonist ATL146e 5 minutes before reperfusion produces a significant reduction in T-cell accumulation and a significant reduction in infarct size (percentage of risk area) measured at 24 hours. In Rag1 KO mice lacking mature lymphocytes, infarct size is significantly smaller than in C57BL/6 mice. Infarct size in Rag1 KO mice is increased to the level of B6 mice by adoptive transfer of 50 million CD4+ T lymphocytes derived from C57BL/6 or A2AR KO but not interferon-γ KO mice. ATL146e completely blocked the increase in infarct size in Rag1 KO mice reconstituted with B6 but not A2AR KO CD4+ T cells. The number of neutrophils in the reperfused heart at 24 hours after infarction correlated well with the number of lymphocytes and infarct size.

**Conclusions**—These results strongly suggest that the infarct-sparing effect of A2AR activation is primarily due to inhibition of CD4+ T-cell accumulation and activation in the reperfused heart. (*Circulation. 2006;114:2056-2064.*)

**Key Words:** adenosine • inflammation • myocardial infarction • receptors • reperfusion • T lymphocyte

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**Clinical Perspective p 2064**

Clinically, the presence of a relative lymphocytopenia is an early marker of myocardial infarction (MI). Tissue accumulation of these cells may be detrimental because intact animal studies show that immunodeficient mice have significantly smaller infarcts than wild-type control mice, and inhibition of T cells by cyclosporine A or tacrolimus can protect the heart against I/R injury and significantly suppress neutrophil infiltration. Thus, the activation and sequestration of lymphocytes may contribute to myocardial I/R injury.

Activation of the adenosine A2A receptor (A2AR) reduces reperfusion injury in liver, kidney, skin, spinal cord, and heart. Heart studies using in vivo animal models have shown that A2AR activation by CGS-21680 or ATL146e during reperfusion significantly inhibits neutrophil recruitment in addition to reducing MI. Our previous study demonstrated that the infarct-sparing effect of A2AR activation in mice during myocardial reperfusion is primarily due to its action on bone marrow–derived cells, possibly lymphocytes.

Mature T cells are divided into 3 major types: CD4+ T-helper (Th), CD8+ T-cytotoxic (Tc), and CD4+ T-regulatory (Treg) cells. In the present study we define the kinetics of changes in circulating and cardiac-resident T lymphocytes during early reperfusion after myocardial ische-
mia, and we explore the effects of T-lymphocyte subtypes (CD4 and CD8) in mediating myocardial inflammatory responses. The results indicate that A2AR-mediated protection of the heart is accompanied by a decrease in the rapid accumulation and activation of CD4+ T cells during reperfusion after myocardial ischemia.

**Methods**

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

This study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985) and was conducted under protocols approved by the University of Virginia Animal Care and Use Committee.

**Animals**

This study used a total of 239 (8- to 14-week-old) male mice of different strains, including C57BL/6 (B6) mice, A2AR knockout (KO) mice, Rag1 KO (B6.129S7-Rag1tm1Mom) mice, and IFN-γ KO (B6.129S7-Ifngrtm1Agt) mice. Among these, 78 mice were used as CD4+ T lymphocyte donors to Rag1 KO mice at a ratio of 2 to 1 to create chimeric mice. Nine B6 mice were used to evaluate CD4+ and CD8+ T-lymphocyte populations after treatment with antibodies by flow cytometry. The remaining mice (B6, Rag1 KO, and chimeras, totaling 152) were assigned to 16 groups and underwent either myocardial I/R injury (15 groups) or sham operation (1 group) (Table). B6, Rag1 KO, and IFN-γ KO mice were purchased from Jackson Laboratory (Bar Harbor, Me). A2AR KO mice, derived from breeders originally supplied by Dr Jiang-Fan Chen (Harvard Medical School, Boston, Mass), were bred to be congenic with B6 mice with the use of microsatellite-assisted breeding congenics. Some groups of mice were treated with the A2AR agonist ATL146e (Adenosine Therapeutics, LLC, Charlottesville, Va), injected intraperitoneally at a dose of 10 μg/kg 5 minutes before reperfusion. The cardioprotective effects of this compound are entirely due to A2AR activation because protection is absent in mice treated with the A2AR-selective antagonist ZM241385 or lacking the A2AR gene.13

**Chimeric Mice Created by Adoptive Transfer of Lymphocytes**

**Isolation of CD4+ T Lymphocytes by Negative Selection**

Mononuclear spleen cells were generated from murine spleens according to an established protocol.1 Spleen cells at a concentration of 5×10^6 cells per milliliter were then used for CD4+ T-lymphocyte isolation with a SpinSep murine CD4+ kit (Stem Cell Technologies, Vancouver, Wash) according to the manufacturer’s protocol. Greater than 94% of the enriched cells expressed CD4 as assessed by flow cytometry.

**Adoptive Transfer of Lymphocyte Subpopulations**

Rag1 KO mice were anesthetized with pentobarbital (50 mg/kg). The left external jugular vein was exposed with a small skin incision. T cells (5×10^7; in a volume of ~200 μL) were injected. Flow cytometry confirmed effective reconstitution of T lymphocytes in the peripheral blood and spleens of Rag1 KO mice. Five days after the reconstitution, chimeras underwent myocardial I/R injury.

**In Vivo Depletion of CD4+ or CD8+ T Lymphocytes in B6 Mice**

Two groups of B6 mice were depleted of either CD4+ or CD8+ T lymphocytes with the use of selective antibodies as reported by others.1,18 Anti–CD4 monoclonal antibody (GK1.5) or anti–CD8a monoclonal antibody (53 to 6.7) (eBioscience, San Diego, Calif) was injected intraperitoneally on 2 consecutive days at a dose of 0.2 mg/d per mouse. Two days after the second injection, 3 animals from each group were used to test for successful depletion by flow cytometry. The rest underwent myocardial I/R injury.

**Flow Cytometry Analysis**

Whole-blood samples were collected under anesthesia by puncturing the right ventricle. Splenocytes were isolated and purified as de-
scribed above. One million splenocytes (in 100 µL) or 100 µL of whole blood was treated with 1 µg of anti-CD8 fluorescein isothiocyanate–labeled or anti-CD4 phycoerythrin-labeled antibodies in 50 µL phosphate-buffered saline/10% fetal calf serum for 30 minutes on ice. Flow cytometry data acquisition and analysis were performed on a FACSscan with the use of CellQuest software (Becton Dickinson, San Jose, Calif).

**Myocardial I/R**

Mice were subjected to 40 or 45 minutes of coronary occlusion followed by up to 24 hours of reperfusion and then euthanized to count peripheral white blood cells and to evaluate MI size and/or leukocyte infiltration (Table). A standard protocol was used, as detailed previously.13

In the CD4- and CD8-depletion studies, the I/R protocol was modified from 45 minutes/24 hours to 40 minutes/60 minutes. Reperfusion time was reduced from 24 hours to 1 hour to focus the study on early inflammatory events and to increase throughput by reducing time to follow-up. This modification was supported by recent longitudinal cardiac magnetic resonance imaging studies undertaken in the murine model by our laboratory showing no significant increase in infarct size between 1 and 24 hours after reperfusion (data not shown).

**Immunohistochemistry of Neutrophils and CD3+ T Cells**

A standard protocol was used, as detailed previously.13 CD3 was used here as a general marker of T lymphocytes, that is, nearly all CD4+ and CD8+ T cells also express CD3.

**Peripheral Blood Cell Count**

Perioperatively, blood (30 to 40 mL) was obtained by puncturing the left external jugular vein at each time point. Cell counts were performed with a HemaVet Hematology System (CDC Technologies, Oxford, Conn).

**Measurement of Plasma IFN-γ by Enzyme-Linked Immunosorbent Assay**

Plasma levels of IFN-γ were measured according to the manufacturer’s protocol (BD Bioscience, San Diego, Calif).

**Statistical Analysis**

All data are presented as mean±SEM. Infarct sizes and risk region sizes were compared by 1-way ANOVA followed by the Student’s t test for unpaired data with Bonferroni correction among Rag1 KO groups or among B6 groups. Square roots of tissue cell counts were compared by 1-way ANOVA. Serial changes of peripheral leukocytes were analyzed by repeated-measures ANOVA with Bonferroni correction.

**Results**

**Exclusion and Mortality**

Of the 152 mice that underwent myocardial I/R injury, 3 mice died early after left anterior descending coronary artery (LAD) occlusion or reperfusion. Four mice were excluded because of either inordinately small risk regions (<25% of left ventricular [LV] mass, 2 mice) or technical failure of 2-3-5-triphenyl tetrazolium chloride (TTC) or phthalo blue staining (2 mice) (Table).

**Changes in Peripheral Blood Cells and Platelets During Early Reperfusion**

Fifteen B6 mice were divided into 3 groups (5 each) and underwent either sham thoracotomy or 45-minute LAD occlusion followed by 60-minute reperfusion (MI±ATL146e groups). Whole-blood samples were taken before thoracotony, at 40 minutes after LAD occlusion and 60 minutes after reperfusion from mice with MI, and at corresponding time points from sham mice. A total of 150 µL of whole blood were withdrawn from each mouse. No significant changes were found in hemoglobin levels or red blood cell counts throughout the procedures or among the different groups. Normally, the total white blood cell (WBC) count was 4470±450/µL. Lymphocytes were predominant and accounted for 76.5±3.2% of murine WBC, whereas neutrophils accounted for 20.1±3.2% and monocytes for 2.9±0.3%. No significant changes in red blood cells or platelets were found throughout the sham procedures (hemoglobin, 11.7 to 13.6 g/dL; platelets, 660±1×10^3/µL). There was a trend toward leukocytosis at 40 minutes after LAD occlusion and a trend toward leukocytopenia 60 minutes after reperfusion in the MI group, but the changes did not achieve statistical significance.

![Figure 1](http://circ.ahajournals.org/DownloadedFrom/...)

**Figure 1.** Effects of MI on circulating leukocytes. A, The total number of WBC was not significantly changed after MI. B, The number of circulating neutrophils (NE) was significantly elevated during ischemia and reperfusion. C, Circulating lymphocytes (LY) were significantly reduced during reperfusion, and this reduction was blocked by ATL146e (ATL). *P<0.05 vs corresponding baseline; †P<0.05 vs sham or ATL146e at same time point.
(Figure 1A). Circulating neutrophils were significantly increased both during ischemia and during reperfusion in the MI groups (Figure 1B). Significant lymphocytopenia was observed on reperfusion in the MI group. ATL146e, administered 5 minutes before reperfusion, significantly attenuated the reperfusion-induced lymphocytopenia (Figure 1C). These findings suggest that neutrophils are recruited, probably from bone marrow, within 40 minutes of reperfusion after MI. This recruitment is not affected by ATL146e. Lymphocytes in peripheral blood are decreased at 60 minutes after reperfusion, and this effect is blocked by ATL146e (Figure 1C).

**Kinetics of Leukocyte Extravasation Into Myocardium During Early Reperfusion**

Sixteen B6 mice underwent 45 minutes of LAD occlusion and 60 minutes of reperfusion. Four hearts were removed respectively at 2, 15, 30, and 60 minutes after the start of reperfusion. An additional 4 hearts were harvested from sham-operated mice. In sham animals, few (0 to 1) neutrophils or T lymphocytes were observed per high-power field. In mice with myocardial I/R injury, there was a rapid increase in neutrophil infiltration into the ischemic region that was statistically significant within 2 minutes after reperfusion compared with sham animals (shown at 0 minutes; \( P < 0.05 \); Figure 2). Neutrophil accumulation progressed between 2 and 30 minutes after reperfusion. The number of CD3\(^+\) T lymphocytes in the previously ischemic myocardium was also significantly increased within 2 minutes after the initiation of reperfusion, but no further increase was noted during the first hour of reperfusion. In ATL146e-treated mice, the infiltration of both neutrophils and CD3\(^+\) T lymphocytes was significantly reduced compared with control at 15 minutes after reperfusion (Figure 3A).

MI size was also evaluated during early reperfusion. As shown in Figure 3B, there was no significant difference in risk region (percentage of LV mass) between vehicle-treated and ATL146e-treated groups; however, infarct size (percentage of risk region) was significantly reduced in ATL146e-treated mice after 45 minutes of LAD occlusion and 60 minutes of reperfusion.

Plasma IFN-\( \gamma \) at 60 minutes after reperfusion was significantly reduced in ATL146e-treated mice compared with vehicle controls (58\( \pm \)13 versus 166\( \pm \)26 pg/mL; \( P < 0.05 \)), but this was still higher than that in sham controls (15\( \pm \)5 pg/mL; \( P < 0.05 \) versus ATL146e group).

**MI in CD4- or CD8-Depleted Mice**

Flow cytometry confirmed that distinct monoclonal antibodies targeting CD4 or CD8 selectively depleted peripheral blood of CD4\(^+\) or CD8\(^+\) T lymphocytes in B6 mice (Figure 4, top 2 panels) and significantly reduced lymphocytes in spleen (Figure 4, bottom 2 panels). We examined infarct size in control mice and mice depleted of CD4\(^+\) or CD8\(^+\) T cells. After 40 minutes of LAD occlusion and 60 minutes of reperfusion, the risk region (percentage of LV mass) ranged from 32\% to 45\% and was comparable between the 3 groups (\( P = \text{NS} \)). In control mice, which have a normal complement of CD4\(^+\) and CD8\(^+\) T lymphocytes, infarct size was 50.7\( \pm \)2.3 (percentage of risk region). In mice without CD8\(^+\) T lymphocytes, infarct size was 52.4\( \pm \)2.4 and did not differ from that

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Time course of accumulation of leukocytes into the heart after reperfusion evaluated by immunostaining of paraffin sections. Top, The number of neutrophils and CD3\(^+\) T lymphocytes per high-power field is plotted against time. Time 0 sections were obtained from sham-operated mice. Significant accumulation of T lymphocytes was detected 2 minutes after reperfusion, but no further increment was noted during 60 minutes of reperfusion. Significant accumulation of neutrophils was observed at 2 minutes of reperfusion and rapidly increased over time. Bottom, Representative immunohistochemical images of neutrophils and CD3\(^+\) T lymphocytes in the previously ischemic myocardium from each time point during reperfusion. \( * \), \( \dagger P < 0.05 \) vs corresponding sham at time 0.
in control mice (P = NS). However, in mice without CD4+ T lymphocytes, infarct size was significantly reduced to 35.3 ± 3.7, a >30% reduction compared with control or CD8-depleted mice (P < 0.05 versus either group; Figure 5).

**Role of A2ARs on CD4+ T Lymphocytes in Mediating MI**

We next sought to learn more about the mechanisms of T cell–mediated myocardial injury by using adoptive transfer of various T-cell populations into Rag1 KO mice (Table). In B6 control mice, 45 minutes of LAD occlusion created a MI size of 61.0 ± 2.3 (percentage of risk region) evaluated at 24 hours after reperfusion. ATL146e treatment reduced the infarct size to 39.3 ± 4.7 (a 36% reduction from B6 control; P < 0.05).

In Rag1 KO control mice, the infarct size (40.5 ± 3.3) was comparable to that of ATL146e-treated B6 mice but was significantly smaller than that of B6 control mice (P < 0.05). After reconstitution with CD4+ T lymphocytes, infarct size was significantly increased in Rag1 KO mice (to 52.1 ± 1.6%), returning it nearly back to the level of B6 control mice. The increase in infarct size in Rag1 KO mice reconstituted with CD4+ T cells was completely blocked by treating the mice with ATL146e 5 minutes before reperfusion. However, ATL146e failed to block the increase in infarct size induced by adoptive transfer of CD4+ T lymphocytes from A2AR KO mice. Interestingly, there was no significant increase in infarct size in Rag1 KO mice reconstituted with CD4+ T lymphocytes from IFN-γ KO mice, indicating that this cytokine plays an important role in reperfusion injury (Figure 6).

Infiltration of T lymphocytes and neutrophils into the myocardium was evaluated in mice 24 hours after MI. CD3+ T lymphocytes were not detectable in hearts from Rag1 KO mice but accumulated in the previously ischemic myocardium after adoptive transfer of B6 CD4+ cells. This response was blocked by the bolus administration of ATL146e just before reperfusion. Adoptive transfer of CD4+ T lymphocytes from A2AR KO mice also resulted in infiltration of T lymphocytes during reperfusion, but ATL146e failed to block the myocardial accumulation of these receptor-deficient T cells. Only a few T lymphocytes were found in the myocardium of Rag1 KO mice reconstituted with CD4+ T lymphocytes from IFN-γ KO mice (Figure 7, top panel).

The accumulation of neutrophils into the infarcted myocardium 24 hours after MI showed the same pattern of response as the accumulation of T cells. Compared with B6 mice, neutrophils were significantly reduced in the previously ischemic region in Rag1 KO mice, Rag1 KO mice reconstituted with CD4+ T lymphocytes, and ATL146e-treated Rag1 KO mice reconstituted with B6 CD4+ T lymphocytes. ATL146e failed to reduce neutrophil accumulation in the infarcted hearts of Rag1 KO mice reconstituted with A2AR KO CD4+ T lymphocytes (Figure 7, bottom panel).

**Discussion**

Studies using in vivo animal models have shown that A2AR activation by CGS-21680 or ATL146e during reperfusion significantly inhibits neutrophil recruitment and reduces MI.13,15,16 Bone marrow–derived cells are the primary targets of A2AR agonists, but the identity of the specific cell type(s) bearing the A2ARs that are responsible for mediating the beneficial effects of A2AR activation to reduce MI size has not been established. The present study shows that CD4+ but not CD8+ T cells are the primary targets of A2AR agonists and that the rapid (15 minute) accumulation of CD4+ T cells in the heart after MI is inhibited by ATL146e. Although the number of T cells entering the heart within the first 30 minutes of reperfusion after ischemia is small, these cells may participate in an inflammation cascade involving sequential activation of T cells, possibly tissue-resident macrophages, and neutrophils. This cascade appears to require INF-γ release from CD4+ cells because cells lacking this cytokine are protected from MI. It is noteworthy that deletion of IFN-γ from CD4+ T cells not only reduces infarct size (Figure 6) but also reduces both T-cell and neutrophil accumulation in the heart 24 hours after infarction (Figure 7). This suggests that IFN-γ release from CD4+ T cells in the heart participates in attracting additional T cells and neutrophils to the heart at later times. Neutrophil trafficking into the heart is also inhibited by ATL146e, but it appears that the effects of
ATL146e on neutrophil trafficking are largely secondary to effects on T lymphocytes because the A2AR agonist is not protective in mice when the A2ARs are selectively deleted from CD4\(^+\) cells.

The observation that Rag1 KO mice are protected from MI does not in and of itself implicate T cells as regulators of reperfusion injury because these animals also lack B cells and have abnormal innate immune responses. The observation that adoptive transfer of CD4\(^+\)/H\(^1\)1001 cells reconstitutes severe reperfusion injury provides clear evidence that CD4\(^+\)/H\(^1\)1001 T cells can trigger increased injury and inflammation, but this still does not prove that these cells play such a role in wild-type mice. Thus, it was important to confirm the role of CD4\(^+\)/H\(^1\)1001 cells in reperfusion injury with the use of antibody depletion as an independent means of modulating the number of CD4\(^+\) or CD8\(^-\) cells in wild-type mice. Taken together, the CD4/CD8-depletion experiments in B6 mice and the adoptive transfer experiments in Rag1 KO animals provide strong evidence that CD4\(^+\)/H\(^1\)1001 cells play a central role in reperfusion injury to the heart. The results also implicate CD4\(^+\)/H\(^1\)1001 T cells as the principal targets of protection by the A2AR agonist ATL146e. This is based on observations that the rapid accumulation of T cells into the reperfused heart is strongly inhibited by ATL146e and that this T-cell accumulation is not inhibited if T cells lack the A2AR.

We have shown previously that activation of the A2AR on CD4\(^+\)/H\(^1\)1001 T cells can reduce T-cell receptor–mediated INF-\(\gamma\) production by 98%.\(^{19}\) INF-\(\gamma\) is produced by activated T cells and activates antigen presenting cells such as macrophages and dendritic cells. In the present study, additional evidence supporting a role of T cell–derived INF-\(\gamma\) in myocardial reperfusion injury includes the following: INF-\(\gamma\) accumulates in plasma of animals after MI; accumulation of INF-\(\gamma\) is inhibited by ATL146e; T cells from IFN-\(\gamma\) KO mice accumulate in infarcted myocardium to a lesser extent than T cells from B6 mice; and transfer of T cells from IFN-\(\gamma\) KO mice to Rag1 KO mice fails to increase injury. Considered together, the data strongly suggest that A2AR agonists reduce myocardial reperfusion injury largely by their effects on CD4\(^+\)/H\(^1\)1001 T cells.

**CD4\(^+\)/H\(^1\)1001 T Lymphocytes Mediate Inflammatory Responses During Reperfusion**

Immune system–mediated myocardial damage has been implicated in a broad range of cardiac diseases including cardiac transplant rejection and ischemic heart disease.\(^{20,21}\) An in-
creasing body of clinical evidence has shown that MI is associated with T-cell responses as identified by increased serum levels of RANTES (regulated on activation normal T cell expressed and secreted), soluble IL-2 receptor, IFN-γ, and chemokines.22,23 Interestingly, in patients with acute MI, circulating T lymphocytes, especially CD4⁺ T cells, are significantly decreased within 24 hours, whereas there are no significant changes in the B-cell count or in serum IgG, IgA, or IgM levels.24 In fact, the presence of a relative lymphocytopenia is an early marker of MI.12 However, the reason for this lymphocytopenia early after MI is poorly understood, and the effects of lymphocytes on the myocardial inflammatory response early after I/R injury are largely unknown. Myocardial ischemia was found to lead to local lymphocytosis consisting predominantly of Th cells, with modest increases in natural killer and T suppressor/cytotoxic subsets.3

Inhibition of T cells by cyclosporine A and tacrolimus has been shown to protect the heart against I/R injury and to significantly suppress infiltration of the neutrophils6,7 that are often considered to be end-effectors of reperfusion injury. These data are consistent with the present results and suggest that the T-cell receptor participates in T-cell activation during reperfusion injury. Thus, our previous results13 and other indirect evidence from the literature provide evidence of activation and trafficking of T cells to the heart after myocardial I/R injury. The present study confirms that T cells play an important role in regulating the early inflammatory responses to myocardial I/R injury.

In the present study we found that after 45 minutes of LAD occlusion and 60 minutes of reperfusion there is no significant change in total WBC number, but neutrophils increase from an initial fraction of 20% to 49% of total WBC, whereas lymphocytes are reduced from 77% to 36% (Figure 1). During this first hour after reperfusion, lymphocytes and neutrophils migrate to the previously ischemic myocardium (Figure 2) and other peripheral organs such as the lung (data not shown). This pattern of inflammatory response indicates that the mobilization of neutrophils from the bone marrow into the blood more than compensates for the loss of blood neutrophils sequestrated into tissues. In contrast, more lymphocytes are sequestrated by tissues than are released into the bloodstream.

A2ARs have a nonredundant role in the attenuation of inflammation and tissue damage in vivo and are a critical part of a physiological negative feedback mechanism for the limitation and termination of both tissue-specific and systemic inflammatory responses.25 Stimulation of A2ARs mediates immunosuppression by inhibiting the activation of T lymphocytes and neutrophils, predominantly by a cAMP-dependent pathway.25,26 Biochemical studies have shown that murine T lymphocytes express the A2AR that is induced on T-cell activation.19,26 Studies of T helper cell subsets (Th1 and Th2) reveal that lymphokine-producing cells are much
more likely to express A2ARs than are cells that do not produce lymphokines. The immunosuppressive effects of A2AR activation have been confirmed by studies showing that genetic inactivation of the A2AR increases the intensity and prolongs the duration of T lymphocyte–dependent proinflammatory cytokine accumulation and tissue damage. ATL146e attenuates the reduction of circulating lymphocytes after MI, consistent with a role of A2AR activation in the inhibition of lymphocyte homing (Figure 1C). Myocardial neutrophil infiltration is also significantly reduced in Rag1 KO mice; however, neutrophil infiltration is restored by adoptive transfer of CD4+ T lymphocytes into Rag1 KO mice (Figure 7). Taken together, these results suggest that CD4+ T lymphocytes are activated early during reperfusion and contribute to an early inflammatory response that contributes to an inflammatory cascade culminating in neutrophil chemotaxis and myocardial reperfusion injury.

In I/R injury, a lack of microbial antigens suggests that T lymphocytes may become activated through antigen-independent pathways. Antigen-independent mechanisms for T-lymphocyte activation have recently been described, involving IL-12, MCP-1, RANTES, macrophage inflammatory protein (MIP-1), and IFN-γ inducible protein-10 (IP-10). The early expression of B7 costimulatory molecules is observed in ischemic organs with a nonimmune, inflammatory response in the absence of alloantigen. Blockade of T-lymphocyte CD28/B7 costimulation, either with antibodies or by knocking out CD28, significantly inhibits T-lymphocyte activation and reduces I/R injury. An additional mechanism of T-lymphocyte activation may involve reactive oxygen species. Very recent work has shown that reactive oxygen species directly stimulate T lymphocytes through the T-cell receptor. Nitric oxide exerts immunosuppressive effects by inhibiting the proliferation of Th1 and Th2 T cells. Reactive oxygen species decrease the bioavailability of nitric oxide and may thus enhance T-cell activation and proliferation through indirect mechanisms.

Conclusion
The present study implicates CD4+ T lymphocytes as targets of adenosine A2AR-mediated cardioprotection against I/R injury. T lymphocytes accumulate in the previously ischemic region of the myocardium within 2 minutes of reperfusion. This rapid accumulation of T lymphocytes, the extent of reperfusion injury, neutrophil accumulation, and IFN-γ release are inhibited by the activation of A2AR on CD4+ T lymphocytes. The results underscore the importance of CD4+ T lymphocytes as mediators of myocardial reperfusion injury after MI.

Acknowledgments
The authors are grateful to the University of Virginia Cardiovascular Research Center for providing the key facilities to fulfill the present experiments.

Sources of Funding
This work was supported by the University of Virginia Partner’s Award and an American Heart Association Grant-in-Aid to Dr Yang; National Institutes of Health grants R01 HL69494 and R01 HL58582 to Dr French and grant R01 HL37942 to Dr Linden; and the Falk Medical Research Trust.

Disclosure
Dr Linden is the president of Adenosine Therapeutics, LLC. The remaining authors report no conflicts.

References
Recent studies suggest that inflammation is induced early during reperfusion by CD4+ T lymphocytes. CD4+ T lymphocytes become activated early during reperfusion and secrete proinflammatory cytokines that potentiate the inflammatory response. Adenosine A2A receptors (A2ARs) are widely expressed on blood-borne cells (including T lymphocytes, monocytes, neutrophils, and platelets). Stimulation of A2ARs on leukocytes mediates immunosuppression and counteracting this T cell–mediated inflammatory response because tissue injury is always associated with release of inflammatory cytokines and chemokines. The present study clearly shows that exogenous activation of A2ARs on CD4+ T lymphocytes inhibits inflammation and reduces myocardial infarction. The cardioprotective effect of A2AR stimulation is manifest even when the compound is given at the time of reperfusion. This has important clinical implications because it demonstrates that it may be possible to intervene with adenosine derivatives to reduce infarct size in patients with acute myocardial infarction. Thus, the myocardial salvage obtained by conventional clinical approaches (percutaneous coronary intervention or thrombolysis) may be significantly improved by the simple administration of a potent A2AR agonist before reperfusion.

**CLINICAL PERSPECTIVE**

Inflammatory responses have been implicated not only in sepsis but also in shock, trauma, pancreatitis, surgery, and reperfusion injury. The cellular, molecular, and genetic mechanisms underlying inflammation have been investigated extensively. However, the immune mechanisms underlying non–antigen-induced inflammation remain unclear. Compelling evidence from both animal and clinical studies indicates that leukocytes are key factors in reperfusion injury. Reperfusion induces a vigorous inflammatory response that includes neutrophil adherence to reperfused endothelium. Recent studies suggest that inflammation is induced early during reperfusion by CD4+ T lymphocytes. CD4+ T lymphocytes become activated early during reperfusion and secrete proinflammatory cytokines that potentiate the inflammatory response. Adenosine A2A receptors (A2ARs) are widely expressed on blood-borne cells (including T lymphocytes, monocytes, neutrophils, and platelets). Stimulation of A2ARs on leukocytes mediates immunosuppression and inhibits neutrophil oxidative activity by a Gs protein/cAMP–dependent pathway. A2ARs play an important role in counteracting this T cell–mediated inflammatory response because tissue injury is always associated with release of adenosine. The present study clearly shows that exogenous activation of A2ARs on CD4+ T lymphocytes inhibits inflammation and reduces myocardial infarction. The cardioprotective effect of A2AR stimulation is manifest even when the compound is given at the time of reperfusion. This has important clinical implications because it demonstrates that it may be possible to intervene with adenosine derivatives to reduce infarct size in patients with acute myocardial infarction. Thus, the myocardial salvage obtained by conventional clinical approaches (percutaneous coronary intervention or thrombolysis) may be significantly improved by the simple administration of a potent A2AR agonist before reperfusion.
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_Circulation._ 2006;114:2056-2064; originally published online October 23, 2006; doi: 10.1161/CIRCULATIONAHA.106.649244

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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