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The Effect of A_{2A} Adenosine Receptor Activation on C-C Chemokine Receptor 7 Expression in Human THP1 Macrophages During Inflammation

Adrienne J. Williams[1] and Bruce N. Cronstein[1,2,3]

Abstract—C-C chemokine receptor 7 (CCR7) and its chemoattractant agonist CCL21 promote cell migration and expression of pro-inflammatory proteins in an atherogenic environment. Since A_{2A} adenosine receptor activation reduces migration and inflammatory effects, we examined its effect on CCR7 expression and migration. CCR7 protein expression decreased by about a third in macrophages treated with A_{2A} receptor agonist CGS 21680 (p=0.028, n=7) and was reversed with antagonist, although mRNA levels increased twofold (p=0.001, n=3). Furthermore, macrophages treated with CGS 21680 showed a significant decrease in migration (p=0.0311, n=7). These results suggest that A_{2A} adenosine receptor activation not only modulates CCR7 expression in both normal and inflammatory environments but also regulates macrophage migration to CCR7-specific chemoattractants.

KEY WORDS: A_{2A} adenosine receptor; macrophage; chemokine receptor migration.

[1] Division of Translational Medicine, Department of Medicine, New York University School of Medicine, New York, NY 10016, USA
[2] Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA
[3] To whom correspondence should be addressed at Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA. E-mail: Bruce.Cronstein@nyumc.org

ABBREVIATIONS: cAMP, Cyclic adenosine monophosphate; CCL19, C-C chemokine ligand 19; CCL21, C-C chemokine ligand 21; CCR7, C-C chemokine receptor subtype 7; CGS21680, 2-p-(2-carboxyethyl) phenethyllamino-5'-*N*-ethylcarboxamido- adenosine; DMSO, Dimethyl sulfoxide; Epac, Guanine nucleotide exchange factor activated by cAMP; Erk, Extracellular signal regulated kinase; FBS, Fetal bovine serum; GAPDH, Glyceraldehyde phosphate dehydrogenase; IFNγ, Interferon gamma; MAPK, Mitogen-activated protein kinase; Mek, MAP kinase kinase; p38, p38 mitogen-activated protein kinase; PCR, Polymerase chain reaction; PKA, Protein kinase A; Rac, Subfamily of Rho GTPases; RNA, Ribonucleic acid; RPMI-1640, Roswell Park Memorial Institute media; SDS–PAGE, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEM, Standard error mean; SYBR Green, *N*',*N*'-dimethyl-*N*-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-*N*-propylpropane-1,3-diamine; THP1, Human acute monocytic leukemia cell line; TNFα, Tumor necrosis factor alpha; TTBS, Tween/Tris-buffered saline ZM 241385–4-(2-[7-amino- 2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol

INTRODUCTION

Atherogenesis occurs when arterial injury leads to a persistent inflammatory response—inflammatory cells respond and migrate to the area, macrophages take up oxidized low-density lipoprotein and, in the process, become foam cells, undergo apoptosis, and accumulate around the lesion, forming a growing plaque that undergoes ineffective repair and may calcify, becoming a complex plaque [1–4]. Chemokines and their receptors play a role in atherogenesis and arterial repair by regulating the recruitment and function of pro-inflammatory and plaque-destabilizing cells [5–7].

The purine adenosine accumulates during tissue injury or stress and, like chemokines, can modulate immune response and cellular function through its receptor subtype A_{2A} [8–11]. A_{2A} adenosine receptor activation suppresses inflammation by regulating cytokine production and prevents atherogenesis by suppressing cell migration and reducing foam cell formation through reverse cholesterol transport [12–17]. The A2A adenosine receptor also modulates chemokine receptor levels; in particular, it downregulates C-C chemokine

receptor subtype 7 (CCR7) expression and function in T and dendritic cells [18–20]. The attraction of monocytes and macrophages to CCR7's agonists could be used to prevent atherogenesis—if fewer new macrophages migrate into the atherosclerotic plaque, further arterial injury may be prevented.

We hypothesized that A2A adenosine receptor activation modulates CCR7 macrophage expression and migration. We found that while A2A adenosine receptor stimulation increased CCR7 mRNA, it decreased protein expression in both noninflammatory and inflammatory environments. As a result, chemotaxis of cells to CCR7 agonists decreased. We also examined signaling at A2A receptors for regulation of CCR7 receptors and found that, as with other functions, adenosine A2A receptors regulate CCR7 expression by a pathway dependent on PKA and MAPK.

MATERIALS AND METHODS

Materials

The selective A_{2A} receptor agonist CGS21680 and its antagonist ZM 241385 were purchased from Tocris Cookson (Bristol, UK). The selective CCR7 agonists CCL19 and CCL21 and the cytokine IFNγ were obtained from R&D Systems (Minneapolis, MN). Epac agonist 8-pcPT-2′-O-Me-cAMP was purchased from Axxora Diagnostics (San Diego, CA) and PKA inhibitor PKI, corresponding to amino acids 5 through 24, from Promega (Madison, WI). Rac inhibitor 553502 and p38 inhibitor SB202190 were obtained from Calbiochem (San Diego, CA), and Mek inhibitor U0126 from Sigma-Aldrich (St. Louis, MO).

Cell Culture

Human monocytic THP1 cells were purchased from American Type Culture Collection (Manassas, VA). Cells were grown in RPMI-1640 media supplemented with 10% FBS, 1% penicillin and streptomycin, and 1% L-glutamine (Invitrogen, Carlsbad, CA) at 37°C, 5% CO_2. Cells were differentiated to macrophages by incubation with 25 ng/mL phorbol 12-myristate 13-acetate (Sigma-Aldrich) for 48 h.

RNA Isolation and Real-Time RT-PCR

THP1 monocytes were differentiated to macrophages (700,000 cells per milliliter), given fresh media, and treated with combinations of CGS21680 (1 μM and 100 nM) and ZM241385 (10 μM) for 4 h. For inflammatory experiments, cells were pretreated with 500 U/mL IFNγ for 12 to 16 h. For signaling experiments, cells were pretreated for 1 h with PKA inhibitor (10 μM), p38 inhibitor (10 μM), Rac inhibitor (10 μM), or Mek inhibitor (10 μM). Media were then removed, and RNA was extracted using Trizol (Invitrogen) according to the manufacturer's instructions. Of total RNA, 1.5 μg was used from each sample for first-strand, complementary deoxyribonucleic acid synthesis with random hexamer primers using an RNA PCR Core Kit (Applied Biosystems, Foster City, CA).

For quantification by real-time PCR, the following primer pairs were used: CCR7 F: 5′-TGACATGCAC TCAGCTCTTG-3 R: 5′-AGGTTTTCAGTCCCTGTGA C-3′ (60°C, 136 bp). GAPDH F: 5′-AACATCATCCCTG CCTCTAC-3′ R: 5′-CCCTCTTGCTGATGCCAAAT-3′ (58°C, 358 bp). PCR product was detected using SYBR Green and the MxPro 3005P from Agilent Technologies (Cedar Creek, TX). Gene expression was quantified to a standard curve and normalized to GAPDH.

Western Blot Analysis

Monocytes were differentiated to macrophages (1.5 million cells per milliliter), given fresh media, and treated with the appropriate cytokines, agonists, and inhibitors for 48 h. Cells were then lysed using radioimmunoprecipitation assay buffer with protease inhibitor cocktail (Sigma-Aldrich) for 30 min at 4°C, centrifuged at 10,000 rpm for 10 min, and the supernatant recovered. Of protein, 35 μg was separated by 10% SDS–PAGE and electrotransferred to nitrocellulose membranes (BioRad, Hercules, CA). Nonspecific antibody binding was blocked with Superblock T20 (Thermo, Rockford, IL) for 4 h at room temperature. The membrane was incubated with a primary antibody against CCR7 (goat polyclonal from Santa Cruz Biotechnologies, Santa Cruz, CA) overnight at 4°C and then incubated with anti-goat secondary antibody conjugated to alkaline phosphatase (Santa Cruz Biotechnologies) for 1 h at room temperature, after washing with TTBS. Protein bands were visualized using electrochemifluorescence substrate and a Storm Phosphoimager (GE Healthcare). Then, the membrane was stripped and incubated with a primary antibody against beta-actin (mouse monoclonal from Abcam, Cambridge, MA) to be used as an internal, normalizing standard. Band intensity was quantified using Kodak Molecular Imaging software (Eastman Kodak, Rochester, NY).

In Vitro Chemotaxis Cell Migration Assay

Monocytes were differentiated into macrophages, given fresh media, and pretreated with the appropriate cytokines, agonists, and antagonists for 48 h. Two hundred thousand cells were then gently lifted using rubber scrapers and Dispase (Roche, Mannheim, Germany) and added to 5-μm chamber inserts cells from the QCM Cell Migration Assay Kit (Chemicon, Temecula, CA) with fresh media containing 1% FBS, 1% penicillin–streptomycin, and 1% L-glutamine in RPMI-1640 media (Invitrogen); 300 ng/mL CCL19 or CCL21 was added to starved media in the lower chamber as a chemoattractant. Cells were then incubated for 24 h, and contents of the lower chamber were detached, stained with fluorescein, and lysed according to the manufacturer's instructions. Migration was quantified using the Victor^{3}V Plate Reader (Perkin Elmer).

Statistics

Data are reported as the mean±SEM. Differences between treatment groups were analyzed with GraphPad Prism (GraphPad Software, San Diego, CA) using one-way analysis of variance followed by Tukey posttest, paired and unpaired Student's *t* tests. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

A_{2A} Adenosine Receptor Agonist CGS21680 Increases CCR7 mRNA Expression

There was a dose-dependent increase in CCR7 message in macrophages following treatment with the adenosine A2A agonist CGS21680 ($p<0.001$ *vs.* control, $n=3$), and co-treatment with the A_{2A} antagonist ZM241385 abrogated the increase $p<0.01$ *vs.* CGS, $n=3$, Fig. 1a).

Cytokines, which are abundant in atherosclerotic lesions, may affect expression of such genes as A_{2A} adenosine receptor expression in THP1 cells. Indeed, IFNγ diminishes both expression and function of A_{2A} receptors and plays a central role in atherogenesis [13]. We therefore determined whether IFNγ altered A_{2A}-mediated increases in CCR7 mRNA. We were surprised to observe that, unlike other adenosine receptor functions, IFNγ did not alter adenosine A_{2A}-mediated

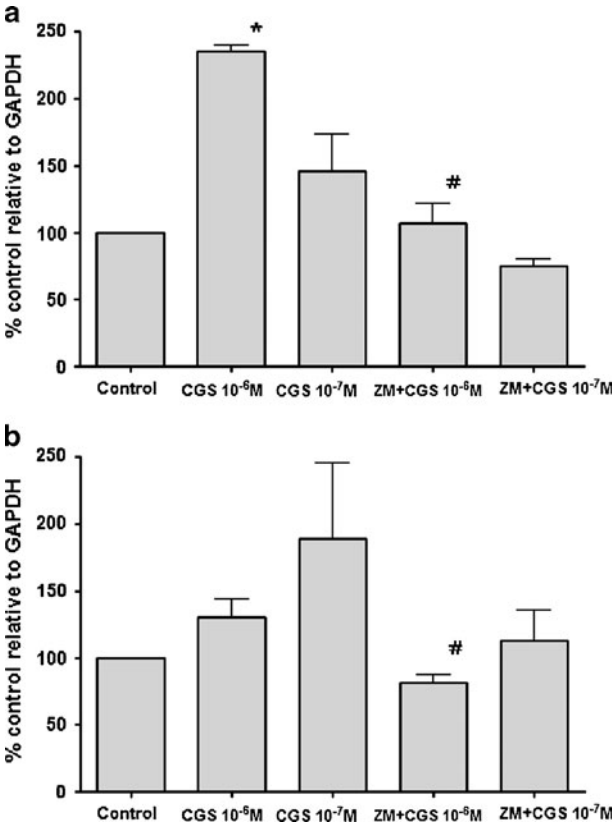

Fig. 1. A_{2A} adenosine receptor agonist CGS21680 increases CCR7 mRNA expression and is reversed with antagonist ZM241385 in THP1 macrophages. Messenger RNA isolated from cells pretreated overnight in **a** regular media conditions or **b** IFNγ (500 U/mL) and then treated with combinations of CGS21680 (10^{-6} M and 10^{-7} M), ZM241385 (10^{-5} M), and DMSO as a negative control for 4 h and were subjected to real-time RT-PCR using specific primers. The expression level was quantified using real-time PCR and normalized to GAPDH. Data shown are the means±SEM of the percentages of control from three independent experiments. **a** *vs.* Control, * $p=0.001$; *vs.* CGS, #$p=0.01$; **b** *vs.* CGS, #$p=0.0382$.

increase in CCR7 message or the capacity of ZM241385 to reverse that effect ($p<0.04$ *vs.* CGS, $n=3$, Fig. 1b).

A_{2A} Adenosine Receptor Agonist CGS21680 Decreases CCR7 Protein Expression

Because A2A receptor stimulation increased mRNA for CCR7, we next determined whether A_{2A} adenosine receptor activation similarly regulated CCR7 protein expression. We were surprised to find that, in contrast to mRNA expression, CCR7 protein levels decreased by about a third ($p<0.03$ *vs.* control, $n=7$), an effect that was

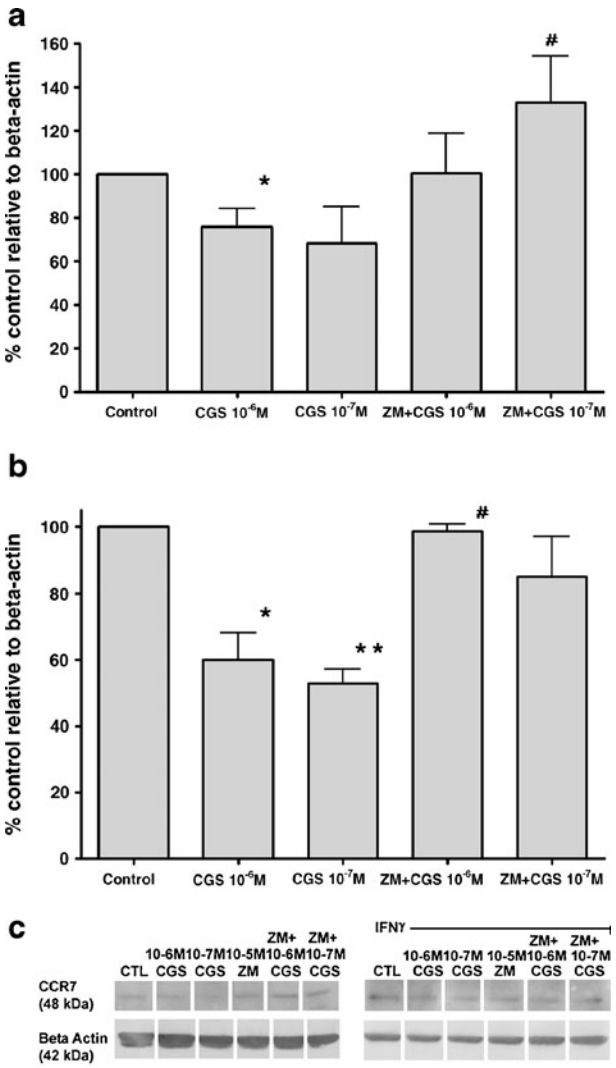

Fig. 2. A_{2A} adenosine receptor agonist CGS21680 decreases CCR7 protein expression and is reversed with antagonist ZM241385 in THP1 macrophages. Cells pretreated overnight in **a** regular media conditions or **b** IFNγ (500 U/mL) and then treated with combinations of CGS21680 (10^{-6} M and 10^{-7} M), ZM241385 (10^{-5} M), and DMSO as a negative control for 48 h had 35 μg of their protein lysate analyzed with SDS–PAGE and immunoblotting. **c** Representative immunoblots. The expression level was quantified using Kodak Image Analysis software and normalized to beta-actin. Data shown are the means± SEM of the percentages of control from **a** seven blots and **b** three blots. **a** *vs.* Control, **p*=0.028; *vs.* CGS, #*p*=0.0432; **b** *vs.* Control, **p*=0.0386, ***p*=0.0085; *vs.* CGS, #*p*=0.0234.

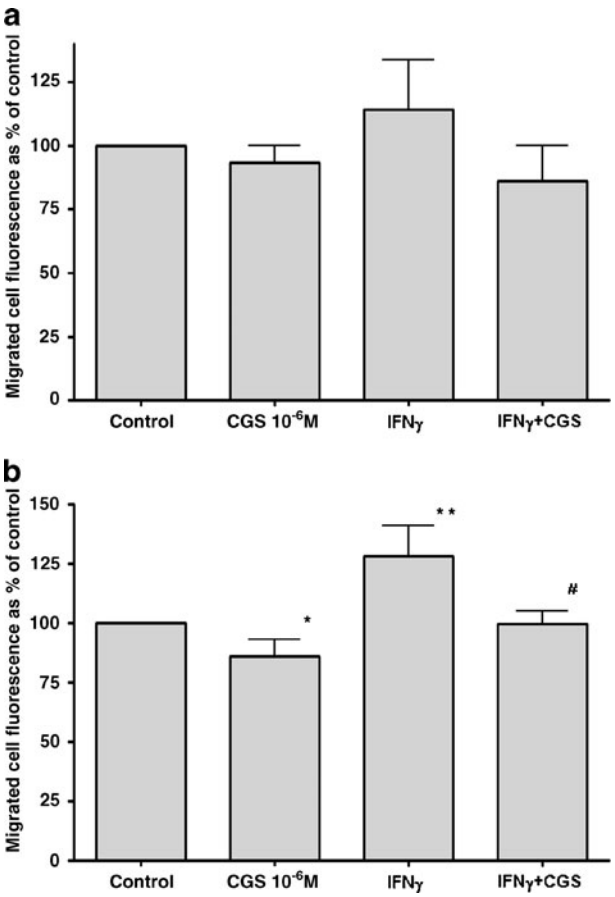

Fig. 3. CGS21680 treatment with pro-inflammatory cytokines decreases THP1 macrophage migration to CCR7 agonists. Cells were pretreated overnight with IFNγ (500 U/mL) or TNFα (10 ng/mL) and then with CGS21680 (10^{-6} M) or DMSO as a negative control for 48 h. Cells were then lifted, added in suspension to 5-μm upper chamber inserts, and migrated to a lower chamber with **a** CCL19 (300 ng/mL) or **b** CCL21 (300 ng/mL) for 24 h. Migration was quantified using fluorescein labeling of cells. Data shown are the means±SEM of the percentages of three independent experiments with CCL19 and six with CCL21. *vs.* Control, * *p*=0.0311, ** *p*=0.0389, *** *p*=0.0196. *vs.* IFNγ, #*p*=0.0273.

reversed by the antagonist ($p<0.05$ *vs.* CGS, $n=7$, Fig. 2a). Pretreatment with the cytokine IFNγ also decreased CCR7 protein levels with CGS21680 treatment ($p<0.04$ *vs.* control, $n=3$) and reversed with ZM241385 ($p<0.03$ *vs.* CGS, $n=3$, Fig. 2b).

CGS21680 Modulates CCR7-Mediated Migration in Normal and Pro-inflammatory Environments

Prior work has indicated that adenosine A_{2A} receptor stimulation retards chemotactic migration to CCR7 ligands by dendritic cells, and our studies indicate

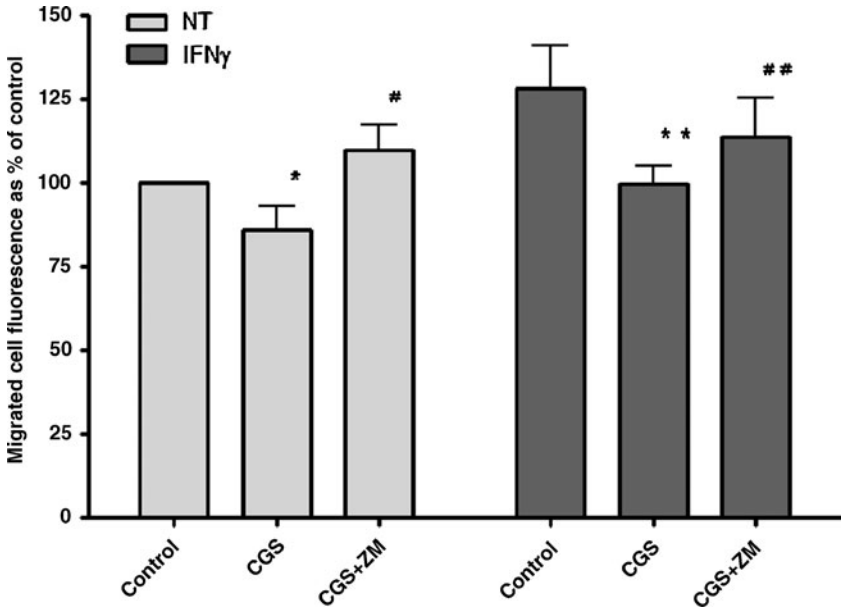

Fig. 4. CGS21680 treatment with and without IFNγ decreases THP1 macrophage migration to CCR7 agonists and is reversed with antagonist ZM241385. Cells were first pretreated overnight with IFNγ (500 U/mL) or TNFα (10 ng/mL) and then with combinations of CGS21680 (10^{-6} M), ZM241385 (10^{-5} M), or DMSO as a negative control for 48 h. Cells were then lifted, added in suspension to 5-μm upper chamber inserts, and migrated to a lower chamber with CCL21 (300 ng/mL) for 24 h. Migration was quantified using fluorescein labeling of cells. Data shown are the means±SEM of the percentages of six independent experiments—three with ZM. *vs.* Control, *p=0.0311, **p=0.0273. *vs.* CGS, #p=0.0078, ##p=0.125.

that adenosine A_{2A} receptor stimulation diminishes CCR7 expression on THP-1 cells [18]. We therefore determined whether adenosine A_{2A} receptor stimulation regulates chemotaxis to CCR7 ligands by THP-1 cells in the absence or presence of inflammation. CGS21680 pretreatment reduced migration by 17% to CCL21 (p<0.03 *vs.* control, n=7) and by a similar amount after IFNγ pretreatment (p<0.03 *vs.* IFNγ, n=7, Fig. 3). Migration to CCL19, a ligand for CCR7, was also reduced. As with the effects on CCR7 expression, the effects of the adenosine receptor agonist were reversed by the A_{2A} receptor antagonist ZM241385 (p<0.008 *vs.* CGS, n=7, Fig. 4).

A_{2A} Adenosine Receptor-Induced Changes in CCR7 Expression Are Blocked with PKA, p38, and Mek Inhibitors

A_{2A} adenosine receptors stimulate adenylate cyclase activity and increase cellular cAMP content, which activates Epac and/or PKA, which activate signaling pathways including MAPK and p38 [21, 22]. To probe the signaling pathways involved, we used pharmacologic inhibitors of protein kinase A and erk1/2 and p38 MAP kinases and observed their effect on the capacity of adenosine A_{2A} receptor stimulation to alter mRNA levels for CCR7. We found inhibitors of PKA and Mek, but not a p38MAPK inhibitor or an inhibitor of rac (Fig. 5a, b). In contrast, p38 and rac inhibition completely blocked the effect of A_{2A} receptor stimulation on CCR7 protein expression (p<0.05 *vs.* CGS with p38 inhibition, n=4, Fig. 5c, d).

DISCUSSION

Since A_{2A} adenosine receptor stimulation reduces inflammatory and atherogenic effects and CCR7 activation promotes these functions, we hypothesized that A_{2A} adenosine receptor activation could reduce CCR7 expression and thus modulate macrophage migration to inflamed sites or atherosclerotic plaques. Our results provide very interesting results regarding

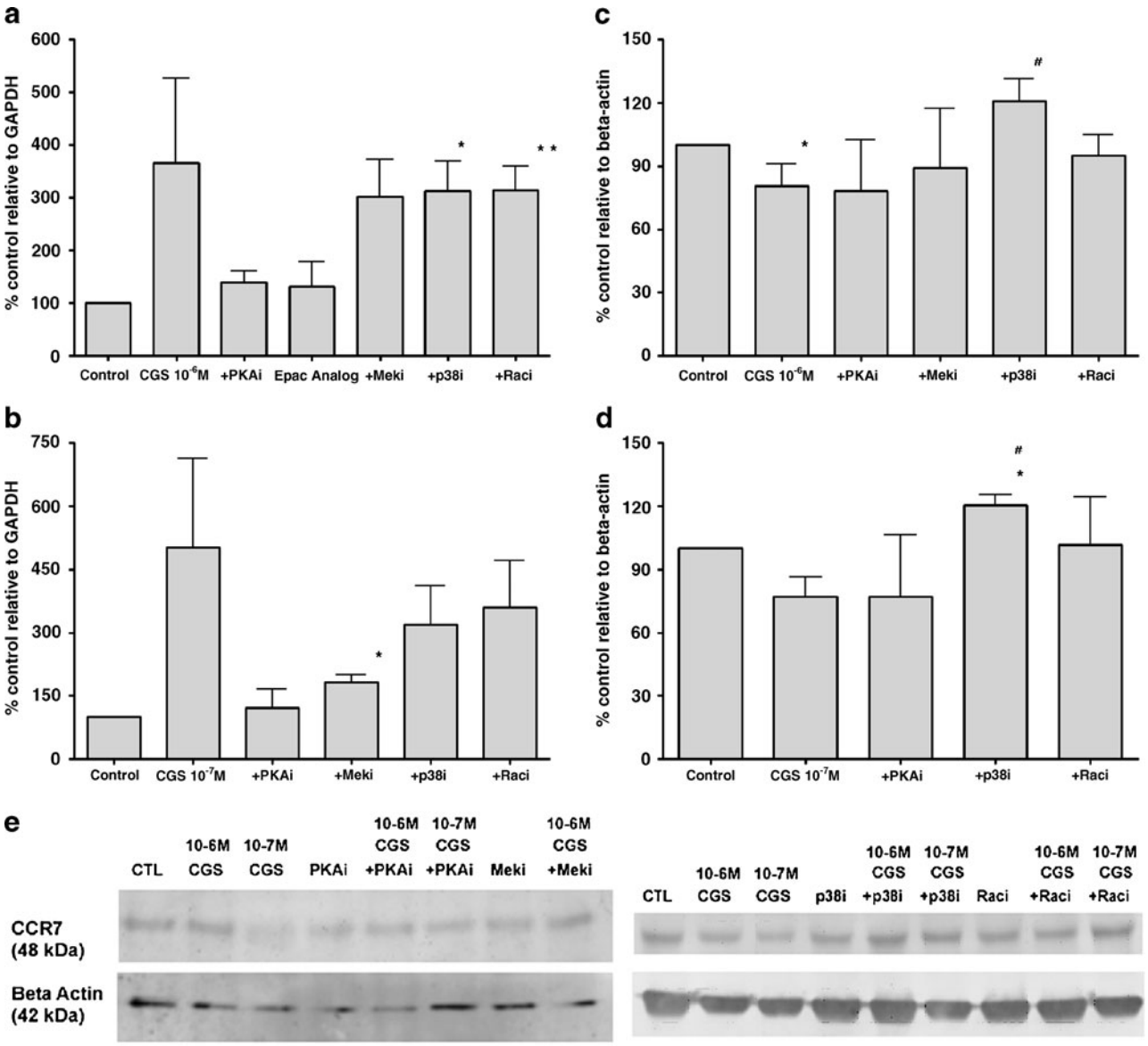

Fig. 5. A_{2A} adenosine receptor-induced increase of CCR7 mRNA expression and decrease of CCR7 protein expression in THP1 macrophages can be reversed with PKA, p38, and Mek inhibitors. Cells were treated with combinations of **a** 10^{-6} M or **b** 10^{-7} M CGS21680 with p38i (10 μM), PKAi (10 μM), Meki (10 μM), Raci (10 μM), Epac analog (10 μM), and DMSO as a negative control for 4 h, and the messenger RNA collected and subjected to real-time RT-PCR using specific primers. The expression level was quantified using real-time PCR and normalized to GAPDH. Data shown are the means±SEM of the percentages of control from three independent experiments. Cells were also treated with combinations of **c** 10^{-6} M or **d** 10^{-7} M CGS21680 and inhibitors for 48 h and had 35 μg of their cell lysate analyzed with SDS–PAGE and immunoblotting. **e** Representative immunoblots. The expression level was quantified using Kodak Image Analysis software and normalized to beta-actin. Data shown are the means± SEM of the percentages of control from three to four blots. **a** *vs.* Control, **p*=0.065, ***p*=0.0416; **b** *vs.* Control, **p*=0.043; **c** *vs.* Control, **p*=0.038; *vs.* CGS, #*p*=0.0504; **d** *vs.* Control, **p*=0.0581; *vs.* CGS, #*p*=0.0204.

the effects of A_{2A} receptor ligation on CCR7 expression. We found that receptor ligation increases CCR7 mRNA by a PKA- and MEK-dependent pathway but diminishes surface expression of the protein by a p38MAPK-, rac-dependent pathway (Fig. 6). Clearly, the resulting reduction in CCR7 chemotactic function results from the diminished receptor expression/function.

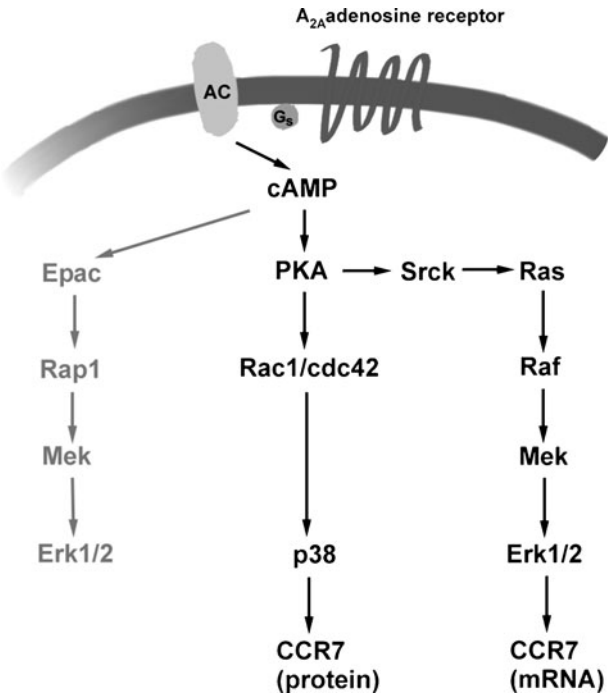

Fig. 6. The A_{2A} adenosine receptor uses signaling pathways downstream of PKA to regulate CCR7 expression. The A_{2A} adenosine receptor subtype's G-stimulatory protein subunit (G_s) activates adenylate cyclase (*AC*), which leads to production of cAMP and stimulation of primarily p38 signaling to decrease CCR7 protein and primarily MAPK signaling to increase CCR7 mRNA in THP1 macrophages.

Macrophages near or within an atherosclerotic plaque are exposed to inflammatory cytokines like IFNγ, which diminish A_{2A} adenosine receptor expression and function and might influence their ability to regulate CCR7 levels [13, 23]. Furthermore, reverse cholesterol transport experiments demonstrate that A_{2A} adenosine receptor function in macrophages is altered by IFNγ [12, 15, 17, 24]. Unlike other adenosine A_{2A} receptor-mediated effects, the A_{2A} receptor-stimulated effects on CCR7 mRNA and protein expression were unaffected by IFNγ.

The contrast between adenosine receptor-stimulated increases in CCR7 mRNA and decreases in CCR7 protein expression and function were surprising. One explanation for these observations is that chemokine receptor processing and transport via the endoplasmic reticulum and Golgi apparatus may be disrupted, degraded, or downgraded by adenosine A_{2A} receptor stimulation. Adenosine A_{2A} receptor stimulation may also increase the rate at which CCR7 receptors are cleared from the surface of the cell, as previously demonstrated for other chemotactic receptors in neutrophils [25–27]. Alternatively, translation of CCR7 message may be blocked by adenosine receptor-stimulated microRNAs; a database search has found some miRNAs with sequence similarity of more than 65% with CCR7, although they are not located in the 5′ region considered most effective for translational blocking (mirbase.org) [28–31].

To determine a signal transduction pathway between A_{2A} adenosine receptor activation and CCR7 expression, we tested pharmacologic signal transduction inhibitors corresponding to previously demonstrated adenosine A_{2A} receptor signaling pathways. A_{2A} adenosine receptor stimulation can activate either the signaling proteins Epac or PKA [17, 32, 33]. PKA activation may then influence many possible downstream pathways, most commonly Erk with its upstream protein Mek and p38 with its upstream protein Rac [21, 34, 35]. We found PKA inhibition reversed A_{2A} adenosine receptor-induced changes in CCR7 message, while Epac analog treatment failed to mimic expression, indicating a PKA-dependent pathway. Mek inhibition blocked change in CCR7 expression as well, while p38 and Rac inhibition did not affect CCR7 message but did change CCR7 protein expression. These results suggest A_{2A} adenosine receptor activation in THP1 macrophages leads to MAPK signaling through a PKA-dependent pathway for CCR7 expression.

Although we showed the presence of IFNγ did not hinder A_{2A} adenosine receptor's ability to reduce CCR7 expression and cell migration, the cytokines TNFα and interleukin-1 also affect A_{2A} adenosine receptor expression but were not examined [13]. A thorough examination of the other two cytokines with CGS21680, or a possible combination of all three, would give a better understanding of the receptor's function in an inflammatory, atherogenic environment.

An increasing body of literature demonstrates that adenosine and its receptors may play a role in the development and regression of atherosclerosis. Prior work demonstrates that adenosine A_{2A} receptor activation diminishes foam cell formation not only by increasing ABCA1 expression but by increasing its function as well [12, 36–39]. We now provide further evidence that adenosine and its receptors might play a beneficial role in atherosclerosis by inhibiting recruitment of macrophages to atherosclerotic plaques.

REFERENCES

1. Lord, R.S., and Y.V. Bobryshev. 2002. Hallmarks of atherosclerotic lesion development with special reference to immune inflammatory mechanisms. *Cardiovascular Surgery* 10: 405–414.
2. Lusis, A.J. 2000. Atherosclerosis. *Nature* 407: 233–241.
3. Steinberg, D. 2002. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nature Medicine* 8: 1211–1217.
4. Takahashi, K., M. Takeya, and N. Sakashita. 2002. Multifunctional roles of macrophages in the development and progression of atherosclerosis in humans and experimental animals. *Medical Electron Microscopy* 35: 179–203.
5. Campbell, D.J., C.H. Kim, and E.C. Butcher. 2003. Chemokines in the systemic organization of immunity. *Immunological Reviews* 195: 58–71.
6. Lucas, A.D., and D.R. Greaves. 2001. Atherosclerosis: role of chemokines and macrophages. *Expert Reviews in Molecular Medicine* 3: 1–18.
7. Zlotnik, A., O. Yoshie, and H. Nomiyama. 2006. The chemokine and chemokine receptor superfamilies and their molecular evolution. *Genome Biology* 7: 243.
8. Bours, M.J., E.L. Swennen, F. Di Virgilio, B.N. Cronstein, and P.C. Dagnelie. 2006. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacology & Therapeutics* 112: 358–404.
9. Hasko, G., P. Pacher, E.A. Deitch, and E.S. Vizi. 2007. Shaping of monocyte and macrophage function by adenosine receptors. *Pharmacology & Therapeutics* 113: 264–275.
10. McPherson, J.A., K.G. Barringhaus, G.G. Bishop, J.M. Sanders, J. M. Rieger, S.E. Hesselbacher, L.W. Gimple, E.R. Powers, T. Macdonald, G. Sullivan, *et al.* 2001. Adenosine A(2A) receptor stimulation reduces inflammation and neointimal growth in a murine carotid ligation model. *Arteriosclerosis, Thrombosis, and Vascular Biology* 21: 791–796.
11. Fredholm, B.B. 2007. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death and Differentiation* 14: 1315–1323.
12. Bingham, T.C., E.A. Fisher, S. Parathath, A.B. Reiss, E.S. Chan, and B.N. Cronstein. 2010. A2A adenosine receptor stimulation decreases foam cell formation by enhancing ABCA1-dependent cholesterol efflux. *Journal of Leukocyte Biology* 87: 683–690.
13. Khoa, N.D., M.C. Montesinos, A.B. Reiss, D. Delano, N. Awadallah, and B.N. Cronstein. 2001. Inflammatory cytokines regulate function and expression of adenosine A(2A) receptors in human monocytic THP-1 cells. *Journal of Immunology* 167: 4026–4032.
14. Link, A.A., T. Kino, J.A. Worth, J.L. McGuire, M.L. Crane, G.P. Chrousos, R.L. Wilder, and I.J. Elenkov. 2000. Ligand-activation of the adenosine A2a receptors inhibits IL-12 production by human monocytes. *Journal of Immunology* 164: 436–442.
15. Reiss, A.B., N.W. Awadallah, S. Malhotra, M.C. Montesinos, E.S. Chan, N.B. Javitt, and B.N. Cronstein. 2001. Immune complexes and IFN-gamma decrease cholesterol 27-hydroxylase in human arterial endothelium and macrophages. *Journal of Lipid Research* 42: 1913–1922.
16. Thiel, M., and A. Chouker. 1995. Acting via A2 receptors, adenosine inhibits the production of tumor necrosis factor-alpha of endotoxin-stimulated human polymorphonuclear leukocytes. *The Journal of Laboratory and Clinical Medicine* 126: 275–282.
17. Block, E.T. 2009. *Interferon gamma inhibits adenosine A2A receptor function in hepatic stellate cells by downregulating expression of adenylyl cyclase in a STAT1-dependent manner.* New York: New York University Department of Basic Medical Sciences.
18. Hofer, S., L. Ivarsson, P. Stoitzner, M. Auffinger, C. Rainer, N. Romani, and C. Heufler. 2003. Adenosine slows migration of dendritic cells but does not affect other aspects of dendritic cell maturation. *The Journal of Investigative Dermatology* 121: 300–307.
19. Sun, J., Y. Zhang, M. Yang, Y. Zhang, Q. Xie, Z. Li, Z. Dong, Y. Yang, B. Deng, A. Feng, *et al.* 2010. Hypoxia induces T-cell apoptosis by inhibiting chemokine C receptor 7 expression: the role of adenosine receptor A(2). *Cellular & Molecular Immunology* 7: 77–82.
20. Zhang, H., Y. Park, J. Wu, X. Chen, S. Lee, J. Yang, K.C. Dellsperger, and C. Zhang. 2009. Role of TNF-alpha in vascular dysfunction. *Clinical Science (London)* 116: 219–230.
21. Fredholm, B.B., Y. Chern, R. Franco, and M. Sitkovsky. 2007. Aspects of the general biology of adenosine A2A signaling. *Progress in Neurobiology* 83: 263–276.
22. Schulte, G., and B.B. Fredholm. 2003. Signalling from adenosine receptors to mitogen-activated protein kinases. *Cellular Signalling* 15: 813–827.
23. Nguyen, D.K., M.C. Montesinos, A.J. Williams, M. Kelly, and B.N. Cronstein. 2003. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. *Journal of Immunology* 171: 3991–3998.
24. Reiss, A.B., S.E. Carsons, K. Anwar, S. Rao, S.D. Edelman, H. Zhang, P. Fernandez, B.N. Cronstein, and E.S. Chan. 2008. Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages. *Arthritis and Rheumatism* 58: 3675–3683.
25. Cronstein, B.N., K.A. Haines, S. Kolasinski, and J. Reibman. 1991. Gs linked receptors (Beta-adrenergic and adenosine A2) uncouple chemoattractant receptors from G proteins. *Clinical Research* 39: 343A.
26. Cronstein, B.N., K.A. Haines, S. Kolasinski, and J. Reibman. 1992. Occupancy of G alpha s-linked receptors uncouples chemoattractant receptors from their stimulus-transduction mechanisms in the neutrophil. *Blood* 80: 1052–1057.
27. Cronstein, B.N., F.R. Rose, and C. Pugliese. 1989. Adenosine, a cytoprotective autocoid: effects of adenosine on neutrophil plasma membrane viscosity and chemoattractant receptor display. *Biochimica et Biophysica Acta* 987: 176–180.
28. The microRNA registry. [mirbase.org]. Accessed 29 June 2010.
29. Hawkins, P.G., and K.V. Morris. 2008. RNA and transcriptional modulation of gene expression. *Cell Cycle* 7: 602–607.
30. Maziere, P., and A.J. Enright. 2007. Prediction of microRNA targets. *Drug Discovery Today* 12: 452–458.
31. Williams, A.E. 2008. Functional aspects of animal microRNAs. *Cellular and Molecular Life Sciences* 65: 545–562.
32. Bos, J.L. 2006. Epac proteins: multi-purpose cAMP targets. *Trends in Biochemical Sciences* 31: 680–686.
33. Wolff, J., C. Londos, and D.M. Cooper. 1981. Adenosine receptors and the regulation of adenylate cyclase. *Advances in Cyclic Nucleotide Research* 14: 199–214.
34. Che, J., E.S. Chan, and B.N. Cronstein. 2007. Adenosine A2A receptor occupancy stimulates collagen expression by hepatic stellate cells via pathways involving protein kinase A, Src, and extracellular signal-regulated kinases 1/2 signaling cascade or p38 mitogen-activated protein kinase signaling pathway. *Molecular Pharmacology* 72: 1626–1636.
35. Hasko, G., J. Linden, B. Cronstein, and P. Pacher. 2008. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nature Reviews* 7: 759–770.
36. Reiss, A.B., K. Anwar, J.T. Merrill, E.S. Chan, N.W. Awadallah, B. N. Cronstein, H. Michael Belmont, E. Belilos, G. Rosenblum, K. Belostocki, *et al.* 2010. Plasma from systemic lupus patients compromises cholesterol homeostasis: a potential mechanism

linking autoimmunity to atherosclerotic cardiovascular disease. *Rheumatology International* 30: 591–598.
37. Reiss, A.B., C.A. Patel, M.M. Rahman, E.S. Chan, K. Hasneen, M.C. Montesinos, J.D. Trachman, and B.N. Cronstein. 2004. Interferon-gamma impedes reverse cholesterol transport and promotes foam cell transformation in THP-1 human monocytes/macrophages. *Medical Science Monitor* 10: BR420–425.
38. Reiss, A.B., M.M. Rahman, E.S. Chan, M.C. Montesinos, N.W. Awadallah, and B.N. Cronstein. 2004. Adenosine A2A receptor occupancy stimulates expression of proteins involved in reverse cholesterol transport and inhibits foam cell formation in macrophages. *Journal of Leukocyte Biology* 76: 727–734.
39. Reiss, A.B., D.W. Wan, K. Anwar, J.T. Merrill, P.A. Wirkowski, N. Shah, B.N. Cronstein, E.S. Chan, and S.E. Carsons. 2009. Enhanced CD36 scavenger receptor expression in THP-1 human monocytes in the presence of lupus plasma: linking autoimmunity and atherosclerosis. *Experimental Biology and Medicine (Maywood)* 234: 354–360.