THE EFFECT OF ADENOSINE ON THE ACTIVATION OF PERIPHERAL CD4$^+$ T-LYMPHOCYTES

Ph.D. Thesis

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INTRODUCTION

Adenosine is a potent endogenous immunosuppressive purine nucleoside, and its anti-inflammatory effects are mediated by its four G-protein-coupled receptors (A_1, A_{2A}, A_{2B} and A_3), which are expressed on the surface of all immune cells. Although resting T-lymphocytes express all four adenosine receptors, previous studies suggested that A_{2A} receptors are the dominant adenosine receptors in governing lymphocyte responses. There is growing evidence that adenosine inhibits the activation and proliferation of naive T-lymphocytes in many experimental systems. However, it is unclear, what role adenosine has in Th1 and Th2 development and how it affects the activation of already polarized, effector Th1 and Th2 cells.

Prolonged or repeated T-cell receptor (TCR) stimulation of previously activated and expanded mature T-cells by an antigen or mitogen causes activation-induced cell death (AICD), which is mediated by the induction of the expression of Fas ligand (FasL) and its interaction with Fas. FasL-dependent cell death plays an essential role in maintaining peripheral tolerance and in limiting an ongoing immune response. Adenosine and its analogues also regulate apoptotic processes, which play an important role in the development and expansion of T-lymphocytes and other cell types. However, prior to our investigations it was unknown, what the possible role of A_{2A} receptors was in regulating TCR ligation-induced, FasL-dependent cell death of mature $CD4^+$ T-cells.

AIMS

We examined the role of A_{2A} receptor activation in regulating the differentiation and effector functions of Th1 and Th2 subpopulations of mouse $CD4^+$ T-lymphocytes using the selective A_{2A} receptor agonist CGS21680. Furthermore, we also studied its importance in activation-induced cell death of T-cells. We were looking for the answers to the following questions:

The role of A_{2A} receptor activation in Th1 and Th2 polarization system

- How does A_{2A} receptor activation affect Th1 and Th2 type cytokine production of T-lymphocytes exposed to *in vitro* Th1 and Th2 polarization?
- How does A_{2A} receptor activation affect proliferation of T-lymphocytes exposed to *in vitro* Th1 and Th2 polarization?

The role of A_{2A} receptor activation in established Th1 and Th2 clones

- How does A_{2A} receptor activation affect Th1 and Th2 type cytokine production of differentiated effector T-hybridomas?
- How does A_{2A} receptor activation affect Th1 and Th2 cytokine mRNA expression of differentiated effector T-hybridomas?
- What is the effect of TCR activation of Th1 and Th2 hybridomas on adenosine receptor expression?

The role of A_{2A} receptor activation in regulation of activation-induced cell death of T-lymphocytes

- What is the effect of A_{2A} receptor activation on the viability of T-lymphocytes?
- How does A_{2A} receptor activation affect activation-induced apoptosis of T-lymphocytes and on related signaling pathways?

METHODS

- Isolation of primary spleen $CD4^+$ cells using MACS technique
- *In vitro* differentiation of primary cells
- Production of immortalized cell lines using hybridoma-technology
- Determination of cytokine concentrations measured by ELISA
- Determination of cell viability using MTT and LDH methods
- Proliferation and apoptosis assays using flow citometry
- Determination of mRNA expression levels using real-time PCR
- SDS-PAGE and Western blot analysis
- Transfection and luciferase activity measurement

RESULTS

The role of A_{2A} receptor activation in Th1 and Th2 polarization system

In our study we examined the effects of A_{2A} receptor activation of Th1 and Th2 cell development and proliferation. Therefore, naive mouse $CD4^+$ T-cells were differentiated *in vitro* under Th1- or Th2-polarizing conditions and restimulated them via TCR activation. For the examination of cell proliferation and expansion, CFSE proliferation assay was performed. In this experiment, $CD4^+$ T-cells derived from WT and A_{2A} receptor KO mice were used. Our results suggest that A_{2A} receptor activation has a strong inhibitory effect on Th1 and Th2 cell responsiveness in the early developmental stage.

- A_{2A} receptor activation inhibits both IFN-γ production of polarized Th1 cells and IL-4, IL-5 and IL-10 production of polarized Th2 cells. This process is regulated via the A_{2A} receptor, because the selective A_{2A} receptor antagonist can restore the inhibitory effect of A_{2A} receptor agonist in both cases.
- A_{2A} receptor activation reduces IL-2 production, and therefore it inhibits the development and proliferation of naive $CD4^+$ T-cells, irrespective of whether they are exposed to Th1- or Th2-polarizing conditions. This effect is not observed in T-cells derived from A_{2A} receptor KO mice.

The role of A_{2A} receptor activation in established Th1 and Th2 clones

We studied the effect of adenosine on the activation of established Th1 and Th2 cell clones. In these experiments, we used mouse T-hybridomas with Th1 or Th2 features derived from *in vivo* immunization and somatic cell fusion. Our results suggest that activation of the A_{2A} receptor has strong inhibitory effects on Th1 and Th2 cell responsiveness in the early developmental stage and the subsequent effector stage as well.

- A_{2A} receptor activation reduces IFN-γ production by activated Th1 cells, and to a lesser extent IL-4 production by Th2 cells. The selective A_{2A} receptor antagonist can restore the inhibitory effect of A_{2A} receptor agonist in both cases.
- A_{2A} receptor activation reduces both IFN-γ mRNA expression of activated Th1 cells and IL-4 mRNA expression of activated Th2 cells.
- Following TCR stimulation, the expression of all adenosine receptors is increased in both Th1 and Th2 cells; however, A_{2A} receptor expression is higher than that of the other receptors in both cases.

The role of A_{2A} receptor activation in regulation of activation-induced cell death of T-lymphocytes

In the present study we demonstrated that the activation of A_{2A} receptor protects $CD4^+$ T-cells from activation-induced cell death (AICD). Apoptosis of Con A-activated T-lymphocytes is mediated by FasL-dependent AICD, as we demonstrated with FasL neutralization assay.

- A_{2A} receptor activation promotes the survival of T-lymphocytes and inhibits their activation-induced apoptosis. The selective A_{2A} receptor antagonist can restore the inhibitory effect of A_{2A} receptor agonist.
- A_{2A} receptor activation protects T-lymphocytes from AICD. A_{2A} receptor activation reduces both early (phosphatidyl-serine translocation to the outer plasma membrane, Fas/FasL expression, caspase-8 cleavage) and late (caspase-3, PARP cleavage, expression of NF-κB, NF-AT, Egr transcription factors) signals of apoptosis.
- The anti-apoptotic effect of A_{2A} receptor activation in T cells is mediated by the regulatory subunit of PKA. Another possible target of cAMP, EPAC is not involved in this process.

CONCLUSIONS

Prior to the current study, it A_{2A} receptors had been implicated in the regulation of lymphocyte responses. The exact role of A_{2A} receptors in regulatingTh1 and Th2 development and activation of Th1 and Th2 effector cells had not been addressed in detail. Our results suggest that activation of A_{2A} receptors has strong inhibitory effects on Th1 and Th2 cell activation in the early (differentiation), as well as in the late (effector) developmental stages.

Furthermore, the present study demonstrates that A_{2A} receptor activation reduces activation-induced cell death of $CD4^+$ T-lymphocytes by down-regulating the expression of both Fas and FasL. While A_{2A} receptors are generally viewed as negative regulators of immune cells, AICD can be viewed as a process that terminates an ongoing immune response of T-lymphocytes. It is, therefore, possible that under certain circumstances that A_{2A} receptor stimulation can actually prolong immune processes. On the other hand, A_{2A} receptor activation may also be anti-inflammatory by promoting the generation of T-cell populations with suppressor activity.

These results suggests that the role of A_{2A} receptors in regulating T-cell-mediated immune responses is more complex then previously thought, and further studies will be necessary to dissect the role of A_{2A} receptors in regulating the immune response. facilitate therapeutic interventions.

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