

# Role of Adenosine in Regulation of T Helper Type 1 and T Helper Type 2 Immunity in Women With Nonpreeclamptic Twin Pregnancies

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## Abstract

**Objective:** This study was to evaluate the relationship between changes in plasma adenosine concentration and T helper (Th1:Th2) cell-immunity in the peripheral blood of women with nonpreeclamptic twin pregnancy.

**Materials and Methods:** Plasma adenosine concentrations and the percentage of Th1, Th2 cells and the Th1:Th2 cell ratios in peripheral blood from 11 normal singleton pregnant women and 11 patients with twin pregnancies without preeclampsia at 28-32 weeks' gestation were measured using high performance liquid chromatography and flow cytometry. The effect of adenosine-receptor blocker 8-sulfophenyltheophylline was also measured *in vitro*.

**Results:** In twin pregnancies, the plasma adenosine concentration and the percentage of Th1 cells were significantly lower than those in singleton pregnancies. After adenosine receptor blockade, there were no measurable differences in the percentage of Th1 cells between twin and singleton pregnancies.

**Discussion:** Our findings suggest the role of adenosine on Th1:Th2 balance toward Th2 dominance in nonpreeclamptic twin pregnancy.

**Keywords:** adenosine, T helper type 1 cell, T helper type 2 cell, twin pregnancy

## Özet

### Preeklamptik Olmayan İkiz Gebeliklerde Adenozinin Yardımcı T-1 ve Yardımcı T-2 Hücresel Bağışıklığının Düzenlenmesine Etkileri

**Amaç:** Bu çalışmada, preeklamptik olmayan ikiz gebeliği olan kadınların periferik kan plazmasındaki adenozin konsantrasyonunun yardımcı T-1/yardımcı T-2 hücresel bağışıklığı (Th1/Th2) arasındaki dengenin değerlendirilmesi amaçlanmıştır.

**Materyal ve Metot:** On bir sağlıklı tekil gebeliğe ve 28.-32. gebelik haftaları arasında olan 11 preeklamptik olmayan ikiz gebeliğe sahip kadın çalışmaya alındı. Yüksek performanslı sıvı kromatografisi ve akım sitometrisi olarak plazma adenozin konsantrasyonları, Th1 ve Th2 yoğunlukları ve Th1/Th2 oranı periferik kandan ölçüldü. Aynı zamanda, adenozin reseptör blokleri olan 8-sülfenilteofilinin etkisi de *in vitro* ölçüldü.

**Sonuçlar:** İkiz gebeliklerde, tekil gebeliklere göre plazma adenozin konsantrasyonları ve Th1 hücrelerinin yoğunlukları anlamlı derecede daha düşüktü. Adenozin reseptör blokajından sonra ikiz ve tekil gebelikler arasında Th1 hücre yoğunlukları arasında ölçülebilir fark saptanmadı.

**Tartışma:** Bulgularımız, preeklamptik olmayan ikiz gebeliklerde adenozinin Th2 lehine olacak şekilde Th1/Th2 dengesindeki etkisini göstermektedir.

**Anahtar sözcükler:** adenozin, yardımcı T hücreleri, ikiz gebelik

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## Introduction

T helper (Th) cells can be divided according to the pattern of cytokines that they release. Th1 cells, which release interleukin (IL)-2, interferon (IFN)- $\gamma$ , and tumor necrosis factor (TNF)- $\beta$ , induce cellular immunity, while Th2 cells, which release IL-4, IL-5, IL-6 and IL-10, induce antibody production (1). Recent studies have suggested that in normal pregnant women, cytokines produced by Th2 cells predominate over those produced by Th1 cells, resulting in the maintenance of pregnancy (2-4). In addition, in our previous study (5) we observed that marked predominance of Th2 immunity occurs in nonpre-eclamptic twin pregnancies because of a significant decrease in the percentage of Th1 cells compared with singleton pregnancies. To date, some possible mechanisms leading to this phenomenon such as estrogen, which is a potent inhibitor of Th1 cytokine production (6), have been discussed.

Adenosine is a vasoactive purine metabolite that is a potent vasodilator in many organs and a metabolic regulator in the cardiovascular system (7). Adenosine also exerts powerful influences on immune-triggered cytokine production and may be speculated to shift the Th1:Th2 balance toward Th2 dominance (8,9). In our earlier study (10), maternal plasma adenosine concentration in nonpre-eclamptic twin pregnancy was significantly higher than that in normal singleton pregnancy. In the current study, therefore, we measured plasma adenosine concentrations and the percentage of Th1, Th2 cells in peripheral blood from women with nonpre-eclamptic twin pregnancy. To clarify the role of adenosine in regulating the Th1:Th2 ratio in twin pregnancy, we also examined the effect of adenosine-receptor blocker 8-sulfophenyltheophylline on Th cells *in vitro*.

## Materials and Methods

In accordance with hospital policies on human research, informed consent was obtained from all subjects prior to the study.

Sodium heparin whole-blood samples were taken from left antecubital veins of 11 patients with a singleton pregnancy at 30.4 $\pm$ 2 (range: 28-32) weeks' gestation and 11 patients with a dichorionic twin pregnancy at 30.2 $\pm$ 2 (range: 28-32; not significantly different from the singleton pregnancy group by Student's *t* test) weeks' gestation without avascularization. A clinical description of the patients is given in Table 1. There were no significant differences in the patients' age by Student's *t* test between the two groups. All the samples were processed immediately after collecting. The eligibility criteria for patients were nulliparity, no smoking, no caffeine intake, no evidence of recent infection, negative C-reactive protein at study, normal response to glucose tolerance testing, no development of preeclampsia during pregnancy, and no treatment with assisted reproductive technology. The gestational age of the pregnancies were established from maternal menstrual history, and confirmed by ultrasonographic examination of the fetal crown-rump length at 9-11 weeks. Chorionicity of the twin pregnancies were diagnosed based on ultrasonic findings and placental pathology. In each

**Table 1.** Clinical description of patients

|   | Singleton pregnancy | Twin pregnancy              |
|---|---------------------|-----------------------------|
| n   | 11                  | 11                          |
| Age (y)   | 29 $\pm$ 5          | 31 $\pm$ 4                  |
| Blood pressure (mmHg)   |                     |                             |
| Systolic  | 114 $\pm$ 7         | 120 $\pm$ 9                 |
| Diastolic   | 70 $\pm$ 6          | 79 $\pm$ 8                  |
| Gestational age (wks)   |                     |                             |
| At study  | 30.6 $\pm$ 2        | 30.2 $\pm$ 2                |
| At delivery   | 39.4 $\pm$ 3        | 36.0 $\pm$ 2*               |
| Neonatal birth weight (g)   | 3011 $\pm$ 210      | 2412 $\pm$ 180 <sup>a</sup> |
| Placental weight (g)  | 468 $\pm$ 45        | 802 $\pm$ 122*              |
| Data are presented as mean $\pm$ SD.                                |                     |                             |
| <sup>a</sup> n=22   |                     |                             |
| *Significant difference ( $p < 0.05$ ) singleton vs. twin pregnancy |                     |                             |

pregnancy, neonatal and placental weights were measured at delivery.

The first 2.0 ml of the blood sample was immediately added to an equal volume of ice-cold stop solution erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride, 120  $\mu$ M; dipyridamole, 20 mM;  $\alpha$ , $\beta$  methylene adenosine-5'-diphosphate, 60 mM; and ethylenediaminetetra-acetic acid dipotassium salt, 4.4 mM) for measurement of plasma adenosine concentration. The mixtures were then centrifuged at 1000 g for 5 minutes at 4°C. The plasma was transferred to an ultrafiltration cone (Amicon; Millipore Corp, Bedford, Mass.) and deproteinized by centrifugation at 6000 rpm for 1 hour at 20°C. Samples of ultrafiltrate were stored at -70°C until analysis using high-performance liquid chromatography using the method previously reported (10,11). Briefly, 50  $\mu$ L of the ultrafiltrate was injected into a C18 column (Radial-Pac; Waters, Milford, Mass. USA) and the absorbance of the eluate was monitored continuously at 254 nm for purine activity.

Flowcytometric determination of IFN- $\gamma$  and IL-4 in the cytoplasm of peripheral CD4<sup>+</sup> T cells was performed by the Fast-Immune cytokine detection system (Becton Dickson Immunocytometry Systems, San Jose, CA, USA) as previously reported (12). The typical forward and side scatter gates for lymphocytes together with CD4<sup>+</sup> gate (logical gate) were set to exclude contaminating monocytes from the analysis. Fifty thousand cells were acquired in the list mode and analyzed with CELL Quest software (Becton Dickinson Immunocytometry Systems). The percentage of IFN- $\gamma$  and IL-4 positive cells (%IFN- $\gamma$  and %IL-4) were counted using FACS (Becton Dickinson Immunocytometry Systems). In this study, CD4<sup>+</sup> Th cell subsets were classified into Th1 (IFN- $\gamma$  positive, but IL-4 negative), and Th2 (IL-4 positive, but IFN- $\gamma$  negative), and the Th1:Th2 cell ratio was evaluated by the ratio of %IFN- $\gamma$  to %IL-4 (IFN- $\gamma$ :IL-4).

Possible changes in the proportion of Th cells were also examined after treatment with a nonspecific adenosine receptor blocker *in vitro* as previously reported (13). Aliquots of

**Table 2.** Th1, Th2 and Th1:Th2 ratio before and after treatment of blood with adenosine receptor blocker (8-sulfophenyltheophylline)

|  | Singleton pregnancy | Twin pregnancy |
|--|---------------------|----------------|
| n  | 11                  | 11             |
| Before administration of 8-sulfophenyltheophylline                       |                     |                |
| Th1 (%)  | 18.9±6.0            | 13.6±5.7*      |
| Th2 (%)  | 2.0±0.8             | 2.1±0.7        |
| Th1:Th2 ratio  | 9.3±3.4             | 6.5±3.1        |
| After administration of 8-sulfophenyltheophylline                        |                     |                |
| Th1 (%)  | 23.1±5.9#           | 19.1±5.0#      |
| Th2 (%)  | 2.1±0.7             | 2.2±0.6        |
| Th1:Th2 ratio  | 10.6±3.4            | 8.9±3.3        |
| Data are presented as mean ± SD.   |                     |                |
| *Significant difference ( $p<0.05$ ) singleton vs. twin pregnancy        |                     |                |
| #Significant difference ( $p<0.05$ ) before vs. after adenosine blockade |                     |                |

heparinized venous blood were incubated with 1 mM of 8-sulfophenyltheophylline (Sigma-Aldrich Japan KK) at 37°C for 15 minutes as an additional incubation. Quantitation of Th1 and Th2 cells was also performed by flow cytometry.

Data are presented as mean ± SD. Statistical comparisons between the two groups were determined by Student’s *t* or Mann-Whitney *U* test.  $P<0.05$  was considered significant.

### Results

The average plasma adenosine concentration in twin pregnancy was  $0.56\pm 0.15 \mu\text{M}$ , which was significantly higher than that in singleton pregnancy ( $0.39\pm 0.13 \mu\text{M}$ ,  $p<0.05$ ).

Results of Th cells and cell ratios of Th1:Th2 before and after treatment of blood with adenosine receptor blocker are shown in Table 2. In twin pregnancies, the percentage of Th1 cells were significantly lower than that in singleton pregnancies. After adenosine receptor blockade, the percentage of Th1 cells increased significantly in both singleton and twin pregnancies. In addition, there were no measurable differences in these increased levels of Th1 cell percentages between the two groups.

### Discussion

To date, the role of increased adenosine in twin pregnancy remains unclear although some hypotheses have been investigated (10,14). In the current study, a significant increase in plasma adenosine and a decrease in the percentage of Th1 cells were observed in peripheral blood from women with nonpre-eclamptic twin pregnancy compared with those in normal singleton pregnancy. These findings are consistent with our previously reports (5,10) and the concept that adenosine is involved in regulation of the Th1:Th2 ratio in twin pregnancy.

A new finding in the current study relates to adenosine blockade using 8-sulfophenyltheophylline. Such blockade was found to increase significantly the percentage of Th1 cells in

both singleton and twin pregnancies and contributed to the disappearance of a significant difference in the percentage of Th1 cells between singleton and twin pregnancies. During pregnancy, the excessive Th1 cell production has been shown to evoke rejection responses directed against fetoplacental semiallografts (15). In addition, adenosine may regulate Th1:Th2 ratio during pregnancy because it has been reported to inhibit IFN- $\gamma$  production by TNF- $\alpha$  and stimulate IL-6 and IFN-10 by Th2 cells (16,17). Thus, the current finding may support the hypothesis that elevated adenosine inhibits the activation of Th1 cells and regulates Th1:Th2 immunity during nonpre-eclamptic pregnancy. In addition, such a role of adenosine may be stronger in twin pregnancy compared to that in singleton pregnancy to minimize the effect of pathophysiologic changes on (feto) maternal circulation in twin pregnancy which are greater than those in singleton pregnancy.

In conclusion, the current findings suggest the role of adenosine in regulating the Th1:Th2 balance toward Th2 dominance in nonpre-eclamptic twin pregnancy.

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