Mifepristone acts as progesterone antagonist of non-genomic responses but inhibits phytohemagglutinin-induced proliferation in human T cells

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BACKGROUND: Progesterone is an endogenous immunomodulator that suppresses T cell activation during pregnancy. The stimulation of membrane progesterone receptors (mPRs) would seem to be the cause of rapid non-genomic responses in human peripheral T cells, such as an elevation of intracellular calcium ([Ca2+]i) and decreased intracellular pH (pHi). Mifepristone (RU486) produces mixed agonist/antagonist effects on immune cells compared with progesterone. We explored whether RU486 is an antagonist to mPRs and can block rapid non-genomic responses and the induction by phytohemagglutinin (PHA) of cell proliferation.

METHODS: Human male peripheral T cell responses in terms of pHi and [Ca2+]i changes were measured using the fluorescent dyes, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) and fura-2, respectively. Expression of mPR mRNA was determined by RT–PCR analysis. Cell proliferation and cell toxicity were determined by [3H]-thymidine incorporation and MTT assay, respectively.

RESULTS: The mRNAs of mPRα, mPRβ and mPRγ were expressed in T cells. RU486 blocked progesterone-mediated rapid responses including, the [Ca2+]i increase and pH decrease, in a dose related manner. RU486 did not block, but enhanced, the inhibitory effect of progesterone on PHA induced cell proliferation. RU486 alone inhibited proliferation induced by PHA and at >25 μM seems to be cytotoxic against resting T cells (P < 0.01).

CONCLUSIONS: RU486 is antagonistic to the rapid mPR-mediated non-genomic responses, but is synergistic with progesterone with respect to the inhibition of PHA-induced cell proliferation. Our findings shine new light on RU486’s clinical application and how this relates to the non-genomic rapid physiological responses caused by progesterone.

Key words: progesterone / mifepristone / RU486 / membrane progesterone receptors / T cells

Introduction

Maternal acceptance of the fetus during pregnancy results in T cell tolerance rather than immunity. However, there is strong evidence to indicate that maternal T cells are not exposed to fetal alloantigens or if exposed, changes in the production of cortisol, progesterone and estrogens play a major role in modulating local immunosuppression at the maternal–fetal interface. These steroids can prevent the maternal immune system from activating the effector T cells capable of attacking the fetal cells and this result in T cell tolerance during pregnancy. The placenta is a major site of progesterone synthesis and hormone concentrations may reach very high levels (1–10 μM in human) (Stites and Siteri, 1983). Progesterone is essential for ovulation and the maintenance of pregnancy as well as for uterine and mammary gland development (Lydon et al., 1995). Progesterone has immunosuppressive effects because it pushes the immune balance from a Th1 type to a Th2 type (Piccinni et al., 1995; Miyaura and Iwata, 2002). Progesterone can protect the pregnant uterus in the striking absence of inflammatory cells and if progesterone is administered during the post-partum period, the absence of inflammatory cells can persist (Padykula and Tansey, 1979).
Mifepristone (RU486) is clinically used in women as an antiprogestosterone in the luteal phase of the cycle to produce early pregnancy interruption (Baulieu and Segal, 1985) and it is also used as an antiglucocorticoid in men (Bertagna et al., 1984). It has been widely used as an antagonist to study the molecular activities of the glucocorticoid and progestosterone receptors. RU486 is defined as a progestosterone type I antagonist because the hormone forms complexes with the progestosterone receptor and its hormonal regulatory elements within DNA (Beck et al., 1996). A single dose of RU486 (200 mg) in women rapidly renders the endometrium unreceptive, which results in endometrial breakdown and menstruation (Danielsson et al., 2003). However, when targeting cells other than reproductive cells, the progestosterone antagonistic activity of RU486 is less clear. For example, an early response to progestosterone withdrawal is a rapid influx of leukocytes such as neutrophils, macrophage, T cells and uterine natural killer cells prior to menstruation itself (Salamonen and Lathbury, 2000).

The rapid non-genomic activities of progestosterone are initiated at the cell surface and have been identified as mediated through non-classical progestin membrane receptors (Falkenstein et al., 2000). The non-genomic blockage of potassium channels by progestosterone inhibits thapsigargin-induced calcium entry, subsequent gene expression and immunosuppression in T cells (Ehring et al., 1998). However, such inhibition of capacitative calcium entry caused by progestosterone is reported to be independent of plasma membrane depolarization (Gamberucci et al., 2004). Progestosterone and immobilization of progestosterone by conjugation to a large carrier molecule, such as bovine-serum albumin (BSA), have been found to inhibit lymphocyte proliferation by a mitogen (Van Voorhis et al., 1989; Chien et al., 2006). The transient intracellular Ca$^{2+}$ ([Ca$^{2+}$]) increase that is stimulated by progestosterone has also been observed in human sperm (Blackmore et al., 1990; Falkenstein et al., 1999). However, impermeable progestosterone (progestosterone-BSA) can mimic the action of progesterone and stimulate rapid non-genomic responses, such as [Ca$^{2+}$], elevation, and intracellular pH (pHi) decrease, and even late responses involving inhibition of the T cell proliferation caused by phytohemagglutinin (PHA). This demonstrates that progestosterone may act directly through T cell membrane specific non-classical progestrone receptors to induce immunosuppression during T cell proliferation (Chien et al., 2006). Recently, membrane progestrone receptors (mPRs) have been found elsewhere, for example coupled to inhibitory G protein (Gi) in human T cells and Jurkat T cells (Dosiou et al., 2008). Therefore, it would be interesting to investigate whether RU486 acts as an antagonist to the mPRs and as such is able to block the rapid non-genomic responses in T cells caused by progestosterone.

Specifically, the suppression of human lymphocyte proliferation by cortisol can be reversed by the antagonist RU486; however, the suppression by progestosterone is not so affected (Van Voorhis et al., 1989). The lack of an antagonist effect of RU486 on progestosterone suggests that progestosterone’s immunosuppressive effects may not be mediated by the cytosolic progestosterone receptor. In this context, it should be noted that nuclear progestosterone receptors have not been consistently identified in T cells (Dosiou et al., 2008). In addition, progestosterone-induced immunosuppression is neither mediated through the classical progestosterone receptors (Kontula et al., 1983; Schust et al., 1996; Henderson et al., 2003) nor mediated by the glucocorticoid receptors (Zskeeres-Bartho et al., 1990). Thus, to date, few studies have investigated the effect of RU486 on T cell proliferation induced by PHA. The aim of present study was therefore to investigate whether RU486 affects the rapid non-genomic response caused by progestosterone, including changes in the [Ca$^{2+}$], and pHi, and the induction by PHA of T cell proliferation.

**Materials and Methods**

**Materials**

Fura-2/AM and 2',7'-bis-(2-carboxyethyl)-5- and 6-carboxyfluorescein (BCECF)/AM were purchased from Molecular Probes (Eugene, OR, USA). PHA, RPMI 1640 medium (RPMI), Hank’s balanced salt solution (HBSS) and fetal calf serum (FCS) were obtained from Gibco (Grand Island, NY, USA). Progestosterone, mifepristone (17β-hydroxy-11β-(4-dimethylamino-phenol)17α-(prop-1-ynyl)estr-4,9,11,13-tetra-one; RU486), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), BSA, ethanol, Ficol/Hypeaque were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [3H]-thymidine was obtained from New England Nuclear (Boston, MA, USA). PHA was dissolved in distilled water. Progestosterone and RU486 were dissolved separately in ethanol. The concentration of ethanol vehicle in the medium was less than 0.1% during all experiments, and the vehicle control was also 0.1% ethanol. The culture medium was supplemented with 10% FCS (v/v). All sera were pretreated with dextran-charcoal to remove small molecules including steroids and thyroid hormone (Liu et al., 1993).

**Preparation of T cells**

Heparinized peripheral blood samples were obtained from 80 healthy male volunteers (20–25 years old) and the blood mononuclear cells (MNCs) were then isolated using the Ficoll-Hypeaque gradient-density method. All volunteers gave written informed consent and this study was approved by the National Yang-Ming University Ethics Committee. The MNC suspension (15 ml) was added to a 100 x 15-mm plastic Petri dish and the cells were incubated for 50 min in a humidified incubator at 37°C, 5% CO$_2$. The adhering cells were harvested using a rubber policeman and washed; this entire process was repeated three times. The non-adhering T cells were prepared by E-rosetting and the rosetted erythrocytes lysed using cold distilled water. At regular intervals, to verify the effectiveness of the separation procedure, the isolated T cells were incubated for 30 min at 4°C with phycoerythrin-labeled monoclonal anti-CD3 antibodies (Ortho Pharmaceuticals, Raritan, NJ, USA) and the antibody-coated T cells separated on a fluorescent-activated cell sorter (EPICS C, Hialeah, FL, USA). The results in all cases showed that the T cell suspension contained almost 100% CD3-positive cells (Chien et al., 2001).

**Measurement of [Ca$^{2+}$]$_i$**

T cells (2 x 10$^6$ cells/ml) were loaded for 30 min at 25°C with fura-2/AM (5 µM) in RPMI 1640 containing 10% FCS (v/v), washed free of extracellular fura-2/AM by three washes with RPMI 1640 and resuspended (4 x 10$^6$ cells/ml) in RPMI 1640 containing 10% FCS. To determine the [Ca$^{2+}$], the T cells (2 x 10$^6$ cells) were then washed twice, resuspended in 2.5 ml of loading buffer (152 mM NaCl, 1.2 mM MgCl$_2$, 1.6 mM CaCl$_2$, 5 mM KCl, 10 mM glucose, 10 mM Hepes, pH 7.4) and placed in a plastic cuvette at 37°C in a dual-wavelength spectrophotometer (Spex Industries, model CM1T11I, Edison, NJ, USA). Using excitation wavelengths of 340 and 380 nm, the fluorescence emission at 505 nm was measured and the [Ca$^{2+}$]$_i$ determined from the fura-2 fluorescence-ratio signal using Spex DM3000 software according to the formula derived by Grynkiewicz et al. (1985).
Measurement of pHi
A T cell suspension (2 x 10⁷ cells/ml) was incubated at 37°C for 30 min with BCECF/AM (3 μM) in HBSS containing 5 mM glucose and 0.2% BSA, then the cells were washed three times with HBSS and resuspended in RPMI 1640 containing 10% FCS. For the pH measurements, 1 x 10⁶ cells were washed twice with HBSS, resuspended in 2.5 ml of the same solution, transferred to a plastic cuvette at 37°C and allowed to stabilize for 15 min before stimulation. Upon excitation at wavelengths of 435 and 500 nm, the BCECF fluorescence emission at 525 nm was measured using a dual-wavelength spectrofluorimeter (Spex Industries, model CM1111, Edison, NJ, USA) and the emission ratio calculated. To prepare the calibration curve, a mixture of 1 x 10⁶ cells and 3 μM nigericin was added to K+ HBSS at pH values of 2–10, then valinomycin (3 μM) was added and allowed to react for 5 min before the fluorescence signals were measured. The pH of the K⁺ HBSS was measured to the nearest 0.001 units using a pH meter (Radiometer Copenhagen, model PHM 93). The calibration values were fitted to a standard sigmoid curve that was then used to calculate the unknown pH values.

Proliferation studies
T cells (2 x 10⁶ cells/ml) were plated in triplicate in a 96-well flat bottom plate (Corning, NY, USA) and stimulated separately with PHA, progesterone or a combination of both. After 72 h, [3H]-thymidine, (specific activity 1 μCi/mM, New England Nuclear, Boston, MA, USA) was added to the wells, the cells were then incubated for a further 18 h and harvested with a Skatron Multwell Harvester (Dynatech, Automash 2000, Billing Shourst, UK). Radioactivity incorporated into the DNA was measured on a multiwell plate reader (PowerWavex 340, BioTek, Winooski, VT, USA). Cell viability was expressed as a percentage of an ethanol control. The results are shown as the mean ± SEM of triplicate cultures.

Cell viability assay
Cell viability was determined by the MTT assay (Mosmann, 1983). A total of 3 x 10⁶ T cells were incubated in 96-well microtiter cell culture plates in the absence (control cells) or presence of increasing amounts of RU486 in a final volume of 100 μl. After 90 h incubation, 10 mM MTT (diluted in 10% sodium dodecyl sulphate/0.01 N HCl and the absorbance at 570 nm was measured on a multiwell plate reader (PowerWave 340, BioTek, Winooski, VT, USA). Cell viability was expressed as a percentage of an ethanol control. The results are shown as the mean ± SEM of triplicate cultures.

RT–PCR
RT–PCR was performed using a programmable thermal controller, Mastercycler personal (Eppendorf, Hamburg, Germany). Total RNA was isolated from human T cells using Trizol reagent and reverse transcribed with Moloney murine leukemia virus reverse transcriptase using random hexamer-mixed oligonucleotides. The complementary DNA (cDNA) was then amplified by PCR in a final volume of 50 μl containing 100 ng cDNA, 5 μl of 10X PCR buffer, each deoxynucleotide 5’-triphosphate at 0.25 mM, each primer at 0.2 μM and 2 U of Taq DNA polymerase (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan). Oligonucleotide primers synthesized by MB Bio, Inc. (Taipei, Taiwan) were identified from the cDNA sequences (Bioman Co, Taipei, Taiwan).

| Table I Primer sequences (5’–3’) used in this study of human male T cells |
|-----------------------------|-----------------------------|
| mPRa (481 bp)               | AATAGAAGCGCAGGTCTGTA        |
| mPRb (289 bp)               | TGGTGCACCCCCCAAGA           |
| mPRy (480 bp)               | GAGATCAGGGGAAACAAAGC        |
| β-actin (764 bp)            | TTGTAACCACTGGGACGATATG      |

mPR: membrane progesterone receptor.

Results
Effects of RU486 on progesterone-stimulated [Ca²⁺]i changes
The dose–response relationship between progesterone and [Ca²⁺]i elevation in T cells was studied as controls for the later experiments. Administration of progesterone (2.5, 5, 10, 20 μM) resulted in an increase in [Ca²⁺]i, starting within 1 min, from a resting level of 95.2 ± 10.4 nM. The increase in [Ca²⁺]i reached a plateau above this resting level of an additional 15.4 ± 2.2 (n = 10, P < 0.01), 23.4 ± 3.6 (n = 10, P < 0.01), 35.8 ± 3.2 (n = 10, P < 0.01) and 46.7 ± 3.5 nM (n = 10, P < 0.001), respectively, at 3 min and these levels continued for 10 min or longer (Fig. 1a).

In order to confirm whether RU486 had blocked this non-genomic rapid elevation in [Ca²⁺], progesterone (10 μM) was used to stimulate [Ca²⁺]i elevation in T cells. The increase in [Ca²⁺]i, caused by progesterone (10 μM) was 42.9 ± 6.7 nM (n = 3) without RU486 pretreatment; after pretreatment of the T cells with RU486 (1, 5, 10 μM) for 30 min, the increase in [Ca²⁺]i by progesterone was reduced to 33.6 ± 4.5 (n = 3, NS), 29.3 ± 3.8 (n = 3, P < 0.05) and 16.8 ± 2.4 (n = 3, P < 0.01), respectively (Fig. 1b).

Effects of RU486 on progesterone-stimulated pHi changes
The dose–response relationships between progesterone and the pHi decrease in T cells were studied. Administration of progesterone...
resulted in a reduction in pHi, starting within 1 min, from a resting level of 7.31 ± 0.08. The reduction reached a steady state of 0.12 ± 0.01 (n = 10, P < 0.01), 0.21 ± 0.02 (n = 10, P < 0.001), 0.36 ± 0.04 (n = 10, P < 0.001) and 0.50 ± 0.06 (n = 10, P < 0.001), respectively, below resting at 5 min after stimulation and these values continued for 10 min or longer (Fig. 2a).

In order to confirm whether RU486 blocked the rapid reduction in pHi by progesterone (10 μM), the dose–response effect of RU486 on progesterone-stimulated pHi reduction in T cells was studied (Fig 2b). The pHi reduction by progesterone (10 μM) was 0.34 ± 0.08 (n = 3). After pretreatment of T cells with RU486 (1, 5, 10 μM) for 30 min; the progesterone-stimulated pHi reductions were 0.19 ± 0.02 (n = 3, P < 0.05), 0.14 ± 0.03 (n = 3, P < 0.01), 0.07 ± 0.03 (n = 3, P < 0.01), respectively.

The effects of RU486 plus progesterone on PHA-induced [3H]-thymidine incorporation in T cells
PHA (1 μg/ml) significantly (n = 5, P < 0.001) increased the degree of proliferation in comparison to the vehicle control as measured by [3H]-thymidine incorporation into T cells. However, when compared with PHA (1 μg/ml) alone, progesterone (1, 5, 10, 25 μM) plus PHA (1 μg/ml) exhibited a significant dose-dependent suppression of the uptake of [3H]-thymidine in T cells (Fig. 3, n = 5).
To verify whether the suppression of T cell proliferation by progesterone can be reversed by its antagonist RU486, co-stimulation with RU486 (1, 5, 10, 50 μM), progesterone (1, 5, 10, 25 μM) and PHA was carried out. Under all these conditions, RU486 was found to exhibit a significant dose-dependent effect with progesterone that suggested a synergistic suppression of PHA-stimulated [3H]-thymidine uptake into T cells (Fig. 4, n = 5).

**Effects of RU486 on PHA-induced [3H]-thymidine incorporation into T cells**

In previous studies, progesterone at 10 μM does not stimulate the uptake of [3H]-thymidine into resting T cells and thus progesterone alone is not capable of inducing proliferation in T cells (Chien et al., 2006). Since RU486 appeared to act synergistically with progesterone to inhibit the uptake of [3H]-thymidine into PHA-stimulated T cells, we tested whether RU486 alone could inhibit the uptake of [3H]-thymidine into PHA-stimulated T cells. After co-stimulation with RU486 (1, 5, 10, 50 μM) and PHA, the results demonstrated that RU486 (at 5 μM and above) had a significant dose-dependent inhibitory effect on the uptake of [3H]-thymidine into PHA-stimulated T cells (Fig. 5, n = 5).

**RU486 decreased the viability of T cells**

In order to confirm whether the inhibition on [3H]-thymidine uptake by RU486 was due to cell toxicity, T cells were incubated for 90 h with...
Resting T cells from three volunteers. Relative levels of mPRs were assessed to determine whether the mRNAs of the mPRs were expressed in these cells. The experiments were carried out on peripheral T cells collected from male human peripheral T cells.

Figure 6 Effects of RU486 on T cell viability.

RU486 (at 1–50 μM) and then cell viability was determined by the MTT assay. A dose-dependent decrease in the number of viable cells was observed (Fig. 6). The IC₅₀ of RU486 was 32.5 ± 1.7 μM for T cells.

Figure 7 Expression of the mRNAs for various mPRs in male human peripheral T cells.

RT–PCR using mPR and β-actin-specific primers was performed on RNA prepared from T cells. Examples of mRNA levels for mPRα (481 bp), mPRβ (289 bp) and mPRγ (480 bp) are shown for three male individuals. β-actin (764 bp) is shown as a control.

Expression of mRNAs for mPRα, mPRβ and mPRγ in male human T cells

The experiments were carried out on peripheral T cells collected from male volunteers with the aim of excluding any effects of endogenous progesterone. In order to confirm that the rapid changes in pH₁ and [Ca²⁺], were mediated through mPRs, it is necessary to know whether the mRNAs of the mPRs mPRα, mPRβ and mPRγ were expressed in male samples. Figure 7 showed the relative levels of mPRα, mPRβ and mPRγ mRNA observed in the resting T cells from three volunteers.

Discussion

In the previous studies of steroids, only progesterone stimulates rapid responses that result in an increase in [Ca²⁺], and a decrease in pH₁ in T cells (Chien et al., 2006). Similar dose-dependent responses for the [Ca²⁺], increase and the pH₁ decrease by progesterone are confirmed here by the controls (Figs. 1a, 2a). It has been shown that pretreatment of T cells with progesterone can inhibit potassium channels, thapsigargin capacitative calcium influx and [Ca²⁺], oscillations that are stimulated by T cell receptor ligation (Ehring et al., 1998). Furthermore, the blockage of potassium channels will depolarize the membrane potential and inhibit receptor-operated calcium channels in Jurkat T cells (Sarkadi et al., 1990). This non-genomic rapid transient [Ca²⁺], increase by progesterone has also been observed in human sperm (Blackmore et al., 1990; Falkenstein et al., 1999). RU486 (10 μM) can counteract the non-genomic effects caused by progesterone in sperm on calcium influx, the acrosome reaction and the penetrative rate into zona-free oocytes (Serres et al., 1994; Yang et al., 1994, 1996). In the present study, RU486 was used to pretreat T cells and then removed to avoid quenching the fluorescence of BCECF and fura-2. The increase in [Ca²⁺], caused by progesterone was observed to be dose-dependently suppressed by RU486 (Fig. 1b). A putative progesterone membrane binding protein has been identified in Chinese hamster ovary cells, and an antibody to this protein can suppress the rapid progesterone-initiated Ca²⁺, increase in sperm (Falkenstein et al., 1999).

Acidification is known to occur in Jurkat T cells under apoptotic CD95 stimulation (Lang et al., 2000). In addition, intracellular acidification can suppress sperm motility, whereas intracellular alkalization promotes [Ca²⁺], increase and causes hyperactivation of sperm (Jones and Bavister, 2000; Marquez and Suarez, 2007). The T cells pretreated with RU486 show dose-dependent suppression of the pH₁ decrease caused by progesterone (Fig. 2b). Both the [Ca²⁺], increase and pH₁ decrease caused by progesterone are recognized as being transmitted by membrane receptors unrelated to the classic intracellular steroid receptors and this activity seemed to be counteracted by RU486. The rapid response in pH₁ reflects that the major early signals induced both by PHA and progesterone are involved; however, PHA stimulates an increase in pH₁ but progesterone stimulates a decrease in pH₁ in T cells. The combination of PHA and progesterone produces acidification of T cells (Chien et al., 2006). This implies that the non-genomic rapid responses on acidification by progesterone might play a role in inhibiting T cell proliferation by PHA. The acidification by progesterone is the result of Na⁺/H⁺ exchange inhibition in T cells (Chien et al., 2007). It has been shown that a cytosolic Ca²⁺, increase by drug treatment does not trigger the release of histamine in mast cells, but cytosolic alkalization does (Pernas-Sueiras et al., 2005).

When progesterone was administered at the same time as PHA, a suppression effect on T cell proliferation was obtained, as was shown in a previous report (Chien et al., 2006) (Fig. 3). The suppression of PHA-induced T cell proliferation can be accomplished by administration of progesterone up to 72 h after PHA stimulation (Chien et al., 2006). This phenomenon indicates that progesterone may act through plasma membrane receptors to block [³H]-thymidine uptake into T cells. In our study, a combination of various doses of RU486 and progesterone were carefully chosen to discover whether
RU486 needs a particular combination of conditions to release the blockage of \(^{3}H\)-thymidine uptake by progesterone. The results in Fig. 4 demonstrated that the progesterone antagonist RU486 could reverse the progesterone non-genomic effects at the membrane sites, and RU486 also appeared to act synergistically with progesterone to inhibit T cell proliferation induced by PHA.

Further studies showed that RU486 alone can inhibit PHA-stimulated T cell proliferation (Fig. 5). As mentioned above, human peripheral blood monocytes and T cells do not possess a classical progesterone receptor (Kontula et al., 1983; Schust et al., 1996; Dosio et al., 2008). Progesterone can inhibit glucocorticoid-induced murine thymocyte apoptosis (McMurray et al., 2000), thus it is possible that the inhibition by RU486 may act through these classical glucocorticoid receptors. RU486 binds to glucocorticoid receptors with an affinity similar to that for progesterone receptors (Mao et al., 2008). RU486 is capable of inhibiting T cell proliferation and acts as an antagonist to progesterone and glucocorticoids. Similar results have been found for the anti-proliferative effects of RU486 on estrogen and progesterone receptor negative MDA-231 cells (Liang et al., 1998).

In addition, RU486 has an antagonistic effect on peroxisome proliferator activated receptor \(\alpha\) nuclear translocation in vascular endothelial cells (Xu et al., 2002). Progesterone at doses greater than 65 \(\mu\)M has significant cytotoxic effects (Stites and Siteri, 1983). We showed that RU486 alone decreased the viability of T cells when employed above 25 \(\mu\)M (Fig. 6), but, when used in combination with PHA, RU486 at 5 \(\mu\)M and above caused an inhibition of T cell proliferation (Fig. 5). Thus, based on the effective dosage of RU486, it is difficult to be certain whether the inhibition of T cell proliferation in the activated T cells was due to cell toxicity or another effect. Therefore, further investigations are required to explore how RU486 inhibits T cell proliferation.

The new family of membrane G protein-coupled progestin receptors has been discovered in spotted seatrout, human, mouse and pig sperm and in T cells from women (Zhu et al., 2003a, b; Luconi et al., 2004; Dosio et al., 2008). In our preliminary study, progesterone-stimulated similar phenomena, namely rapid responses involving an elevation of \(\text{Ca}^{2+}\) and a decrease in \(\text{pH}_i\), in T cells obtained from female volunteers in the follicular phase of a regular menstrual cycle (unpublished data). In the present study, we showed the expression of mRNAs for mPR\(\alpha\), mPR\(\beta\) and mPR\(\gamma\) in male peripheral blood T cells (Fig. 7). This suggests that the stimulation of mPRs may be a general cause of rapid responses in T cells. Recently, it has been shown that only mPR\(\alpha\) and mPR\(\beta\), but not mPR\(\gamma\), were expressed in the T cells of women (Dosio et al., 2008).

Our findings indicated that RU486 is antagonistic to mPR-mediated rapid non-genomic responses, but can enhance the inhibition of PHA-stimulated T cell proliferation by progesterone. RU486 has been shown to block the non-genomic effects of progesterone on acidification and increase in \(\text{Ca}^{2+}\), and therefore RU486 might be used as an important early signal modulator to alter the rapid physiological responses in T cells caused by progesterone. Furthermore, the ability of RU486 to block the non-genomic effects of progesterone suggests that RU486 might play a pivotal role in control of T cells in terms of the Th1/Th2 balance and various inflammatory responses during pregnancy. In conclusion, our findings shine a new light on the clinical application of RU486 and indicate that this drug may affect membrane-linked progesterone receptors, therefore RU486 may be a candidate drug for use in targeted therapy.

## Acknowledgements

We would like to thank Professor Ralph Kirby (Department of Life Sciences & Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan) for his kind assistance in the preparation of the manuscript.

## Funding

This research was supported by grants from the Taipei City Hospital (TCP-96-002-62-078), the National Science Council (NSC95-2320-B-010-029) and Ministry of Education, Aim for the Top University Plan (98A-C-D104), Republic of China.

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Submitted on November 11, 2008; resubmitted on February 4, 2009; accepted on March 20, 2009.