A Nongenomic Mechanism for Progesterone-mediated Immunosuppression: Inhibition of K⁺ Channels, Ca²⁺ Signaling, and Gene Expression in T Lymphocytes

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Summary

The mechanism by which progesterone causes localized suppression of the immune response during pregnancy has remained elusive. Using human T lymphocytes and T cell lines, we show that progesterone, at concentrations found in the placenta, rapidly and reversibly blocks voltage-gated and calcium-activated K⁺ channels (Kᵥ and Kᵥ₉,₉,₉ respectively), resulting in depolarization of the membrane potential. As a result, Ca²⁺ signaling and nuclear factor of activated T cells (NF-AT)-driven gene expression are inhibited. Progesterone acts distally to the initial steps of T cell receptor (TCR)-mediated signal transduction, since it blocks sustained Ca²⁺ signals after thapsigargin stimulation, as well as oscillatory Ca²⁺ signals, but not the Ca²⁺ transient after TCR stimulation. K⁺ channel blockade by progesterone is specific; other steroid hormones had little or no effect, although the progesterone antagonist RU 486 also blocked Kᵥ and Kᵥ₉,₉,₉ channels. Progesterone effectively blocked a broad spectrum of K⁺ channels, reducing both Kᵥ₁.₃ and charybdotoxin-resistant components of Kᵥ current and Kᵥ₉,₉,₉ current in T cells, as well as blocking several cloned Kᵥ channels expressed in cell lines. Progesterone had little or no effect on a cloned voltage-gated Na⁺ channel, an inward rectifier K⁺ channel, or on lymphocyte Ca²⁺ and Cl⁻ channels. We propose that direct inhibition of K⁺ channels in T cells by progesterone contributes to progesterone-induced immunosuppression.

Key words: T lymphocyte • K⁺ channel • calcium signaling • gene expression • nuclear factor of activated T cells

Immunosuppression within the uterus is crucial for the survival of the fetus (1, 2). Although the maternal immune system becomes sensitized to paternal antigens during pregnancy, fetal cells and placental trophoblasts bearing those antigens do not elicit a cytolytic immune response (3–5). High concentrations of progesterone in the placenta inhibit the maternal immune response against the fetal allograft (6, 7). The immunosuppressive effects of progesterone were demonstrated in vivo by prolonged survival of xenografts near silastic implants containing progesterone at concentrations typically found in the placenta (3, 6). In vitro assays have established that progesterone inhibits lymphocyte activation and proliferation in response to allogeneic cells or mitogens (8–10). In contrast, progesterone does not inhibit the effector functions of previously activated cytolytic T cells (11). These data suggest that progesterone may interfere with the early phases of T cell activation.

Antigen presentation and TCR ligation stimulate tyrosine kinases, leading to the generation of inositol 1,4,5-trisphosphate (IP₃) and a consequent rise in the cytoplasmic calcium concentration ([Ca²⁺]ᵢ). Elevated [Ca²⁺]ᵢ activates calcineurin, a phosphatase which then dephosphorylates a cytoplasmic transcription factor, the nuclear factor of activated T cells (NF-AT). Dephosphorylated NF-AT moves into the nucleus where it promotes the expression of the IL-2 gene (12). A sustained elevation in [Ca²⁺]ᵢ, requiring Ca²⁺ influx across the plasma membrane is necessary for the retention of NF-AT in the nucleus and efficient transcription of IL-2 (13–16). In lymphocytes, the opening of Ca²⁺ release-activated Ca²⁺ (CRAC) channels initiates Ca²⁺ influx after the depletion of Ca²⁺ stores by

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IP3 (17–19). Once the CRAC channels are open, the transmembrane concentration gradient for Ca2+ and the membrane potential (Ei) provide the driving force for Ca2+ entry. Ei is set by the interplay between several ion channels in the T cell membrane. By itself, the Ca2+ current through CRAC channels would diminish the driving force for calcium entry by reducing Ei. However, currents through voltage-gated K+ (kv) channels and Ca2+-activated K+ (KCa) channels enhance Ca2+ entry by driving Ei to a negative voltage. Chloride channels may also play a role in maintaining a negative Ei during T cell activation (20, 21). The four major types of ion channels found in T cells are possible targets for immunosuppressive agents. In particular, the kv channel encoded by Kv1.3 is required for normal lymphocyte activation both in vitro and in vivo (14, 22–25).

We have determined the effects of progesterone on lymphocyte ion channels, Ca2+ signaling, and gene expression. By combining functional assays of gene expression with patch-clamp and Ca2+ imaging measurements, we demonstrate that progesterone blocks lymphocyte K+ channels, interferes with TCR-induced [Ca2+]i signaling, and inhibits its gene expression. We propose that progesterone acts as an endogenous immunosuppressant by directly and reversibly blocking K+ channels.

Materials and Methods

Chemicals and Solutions. Salts and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Thapsigargin (TG) was obtained from Alexis Corp. (San Diego, CA). Progesterone (10 mM) and PMA (3 mM) stocks were prepared in DMSO.

Cell Culture. B3Z cells and K897 cells were provided along with the antigenic peptide fragment SIIN FEKL by Dr. N. Shastri (University of California, Berkeley, CA). B3Z cells are a murine, CD8+, T cell hybridoma with a known antigen specificity for OVA/Kb-MHC and containing a β-galactosidase reporter gene construct (laz2) under the control of the NF-AT promoter (26). The corresponding antigen-presenting K897 cells had been transfected with Kβ class I MHC as described (27). The human leukemia T cell line Jurkat E6-1 was obtained from the American Type Culture Collection (Rockville, MD). Human peripheral T lymphocytes were collected from venous blood of healthy volunteers and isolated using a Ficoll-Hypaque density gradient as described previously (28). A population of activated T cell blasts was prepared in culture by treating the resting cells with 10 μg/ml PHA (PHA-P; Difco Laboratories, Inc., Detroit, MI). Cell lines expressing the cloned Kv channels Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, and Kv3.1 and a voltage-gated Na+ channel HSKM1 were maintained in culture. All cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 10 mM Hepes, 2 mM glutamine, 1 mM pyruvate, 50 μM 2-ME. Cells were cultured in 25–ml flasks (Costar Corp., Cambridge, MA) at 37°C, 5% CO2 in a humidified incubator. For experiments on inward rectifier K+ channels, rat basophilic leukemia (RBL) cells were maintained in Eagle’s MEM supplemented with 10% FCS and 1% l-glutamine. RBL cells were plated onto glass coverslips 1–2 d before use.

Laz2 reporter gene assay. The fraction of B3Z cells expressing β-galactosidase was measured using flow cytometry (FAC-Scan; Becton Dickinson, San Jose, CA) as described previously (13). In brief, 5 × 104 cells in RPMI without serum were placed in individual wells of 24-well plates activated either by 1 μM TG plus 50 mM PMA or by antibodies to the CD3-ε subunit of the TCR complex. In the latter, wells were coated with 10 μg/ml anti-CD3-ε antibodies (Pharmingen, San Diego, CA) overnight and rinsed briefly with PBS before use. Cells were activated in the incubator for a total of 4 h before being resuspended and loaded by osmotic shock with the fluorogenic substrate, fluorescein-di-β-galactopyranoside (FDG; Molecular Probes, Inc., Eugene, OR). The fluorescence of laz2 cells was at least fivefold greater than autofluorescence. The effect of progesterone on laz2 gene expression was quantified using a MUG (4-methylumbelliferyl β-d-galactopyranoside) assay (29). In brief, B3Z cells were plated at 105 cells per well in 96-well plates, and the fluorescence produced by cell lysates in a solution containing 3 mM MUG was measured in a multi-well plate reader (CytoFluor Series 4000; PerSeptive Biosystems, Framingham, MA).

Patch-clamp recordings. Membrane currents were measured using the whole-cell configuration of the patch-clamp technique (28, 30), and membrane potential was measured using the perforated patch method in current-clamp mode with nystatin to permeabilize the cells (31). An EPC-9 amplifier (HEKA, Lambrecht/Pfalz, Germany) interfaced to a Macintosh Quadra 700 computer was used for pulse application and data recording. Membrane voltages were corrected for liquid junction potentials, and current recordings were used for pulse and capacitive currents. Patch pipettes were pulled from Accu-fill 90 Micropets (Becton Dickinson, Parsippany, NJ) using a P87 micropipette puller (Sutter Instruments Co., Novato, CA). Pipettes were coated with Sylgard (Dow Corning Corp., Midland, MI) and heat polished to final resistances of 2–5 MΩ. Patch-clamp experiments were performed at room temperature (20–25°C). Unless otherwise indicated, the membrane currents were filtered at 1.5 kHz. Data analysis was performed using the program Pulse (HEKA). Mammalian Ringer contained (in mM): 160 NaCl, 4.5 KCl, 2 CaCl2, 1 M gCl2, 10 Hepes (pH 7.4; osmolality 290–310 mosmol/kg). The ionic composition of the pipette solutions used in the individual experiments is reported in the figure legends.

[Ca2+]i measurement. [Ca2+]i was measured ratiometrically using fura-2, as described previously (13). In brief, cells were loaded with 3 μM fura-2/AM (Molecular Probes, Inc.) for 30–40 min at room temperature (20–25°C). The cells were then washed three times with RPMI 10% FCS and stored in the dark. Illumination was provided by a xenon arc-lamp (Carl Zeiss, Jena, Germany) and transmitted through a filter wheel unit (Lambda 10; Axon Instruments, Inc., Foster City, CA) containing 350- and 385-nm excitation filters. The filtered light was reflected by a 400-nm dichroic mirror through a 63× oil-immersion objective to illuminate cells. Emitted light >480 nm was received by a SIT camera (C2400; Hamamatsu Photonics, Bridgewater, NJ) and the video information relayed to an image processing system (Videoprobe; ETM Systems, Petaluma, CA). Full field-of-view 8-bit images, averaged over 16 frames, were collected at 350- and 385-nm wavelengths. Digitally stored 350/385 ratios were averaged over 16 frames. Images were constructed from background-corrected 350- and 385-nm images. Single-cell measurements of [Ca2+]i were calculated from the 350/385 ratios using the equation of Grynkiewicz et al. (32) and a Kd of 250 nM for fura-2. The minimum 350/385 ratio was measured in single cells after incubation for 10 min in Ca2+-free Ringer containing 2 mM EGTA. Maximum ratio values were obtained after perfusion with Ringer containing 10 mM CaCl2, 1 μM TG, and 10 μM lonomycin.
Results

Progesterone Suppresses Gene Expression Driven by NF-AT. The murine T cell hybridoma, B3Z, recognizes an octapeptide fragment from ovalbumin (SIINFEKL) and expresses the lacZ reporter construct under transcriptional control of the NF-AT response element of the IL-2 promoter (26, 27). Several treatments that increase [Ca\textsuperscript{2+}], lead to NF-AT-driven lacZ expression in B3Z cells (13), including TCR engagement or stimulation with TG, a specific inhibitor of the endoplasmic reticulum Ca\textsuperscript{2+}-ATPase (33). By flow cytometry in the present series of experiments, cross-linking the TCR with anti-CD3-e antibodies or stimulating with TG plus PMA produced lacZ expression in the majority of B3Z cells (60 ± 4%, n = 7; or 72 ± 10%, n = 3, respectively). Progesterone reduced NF-AT-mediated lacZ gene expression in a concentration-dependent manner, with an IC\textsubscript{50} value of 22 ± 2.1 μM in cells stimulated by TG/PMA (Fig. 1A, filled circles). The progesterone antagonist RU 486 also inhibited gene expression with slightly lower potency (Fig. 1A, open squares). Progesterone reduced lacZ expression when B3Z cells were stimulated by TG alone or with PMA, by immobilized anti-CD3-e antibody, or by antigen presentation (Fig. 1B). Thus, at concentrations normally obtained in the placenta (34, 35), progesterone inhibits NF-AT-mediated gene expression when driven by four treatments that increase [Ca\textsuperscript{2+}]. Our results with NF-AT-driven lacZ reporter gene expression are consistent with levels of progesterone or RU 486 shown previously to inhibit activation of human T cells in vitro (8, 9).

Progesterone Inhibits [Ca\textsuperscript{2+}], Signals in T Cells after TCR Engagement or TG Stimulation. Upon contact with K897 cells preloaded with SIIN FEKL, B3Z cells responded with an initial Ca\textsuperscript{2+} transient from a resting [Ca\textsuperscript{2+}] of 180 ± 86 nM to a peak of 2.5 ± 0.5 μM (n = 20), followed by sustained Ca\textsuperscript{2+} oscillations (Fig. 2A). In the absence of preloaded antigen, K897 cells did not elicit Ca\textsuperscript{2+} signaling in B3Z cells (data not shown). Application of 50 μM progesterone reversibly suppressed antigen-induced Ca\textsuperscript{2+} oscillations (Fig. 2A).

To determine if progesterone directly inhibits TCR-initiated signaling or interferes with costimulatory pathways, we activated the TCR complex by cross-linking CD3. Setting B3Z cells onto chambers coated with anti-CD3-e antibodies elicited an initial Ca\textsuperscript{2+} transient followed by vigorous Ca\textsuperscript{2+} oscillations that continued for at least 40 min (Fig. 2B). In the presence of 50 μM progesterone, most cells produced only the initial Ca\textsuperscript{2+} transient lasting 271 ± 178 s, or a transient followed by severely attenuated oscillations (n = 14; Fig. 2C). These data demonstrate that progesterone blocks Ca\textsuperscript{2+} signaling after TCR engagement. Since the initial Ca\textsuperscript{2+} transient results from IP\textsubscript{3}-mediated release of Ca\textsuperscript{2+} from intracellular stores, and the sustained Ca\textsuperscript{2+} signal requires Ca\textsuperscript{2+} influx, these data also suggest that progesterone inhibits Ca\textsuperscript{2+} influx but not the steps that lead to Ca\textsuperscript{2+} release from intracellular stores.

TG inhibits the Ca\textsuperscript{2+} reuptake pump, leading to depletion of the intracellular Ca\textsuperscript{2+} stores and Ca\textsuperscript{2+} influx while bypassing the initial steps of TCR signaling and IP\textsubscript{3} generation (36, 37). In resting human T cells, the addition of TG to the bathing solution increased [Ca\textsuperscript{2+}], from 72 ± 8 nM to a stable plateau level of 1.2 ± 0.1 μM (n = 76; Fig. 3A). Progesterone reversibly inhibited the sustained Ca\textsuperscript{2+} signal with an IC\textsubscript{50} value of 28 ± 2.7 μM (Fig. 3, A and B).

Figure 1. Progesterone inhibits NF-AT-mediated gene expression in B3Z cells (A). The concentration-dependent inhibition of lacZ expression in B3Z cells by progesterone (●) or RU 486 (□) was measured in a multi-well fluorescence plate reader using MUG as a substrate for β-galactosidase. The cells were stimulated for 4 h with 1 μM TG plus 50 nM PMA. For each experiment, triplicate samples were corrected for background fluorescence and normalized for control lacZ expression. Data are presented as mean ± SD (n = 10), and were fitted to a Hill equation of the form

\[
y = \frac{y_{\text{max}}}{1 + \left(\frac{X}{IC_{50}}\right)^n}
\]

where y = the fraction of control lacZ expression with a maximum level represented by y\textsubscript{max}, [X] = the concentration of progesterone, IC\textsubscript{50} = the dissociation constant, and n = the Hill coefficient. The curve represents a Hill equation with an IC\textsubscript{50} value of 22 ± 2.1 μM and n = 1.7 ± 0.3. The effects of progesterone were not due to nonspecific toxicity; since after treatment with 30 μM progesterone, >95% of the cells stained with vital stain acetoxy-methoxy-calcein and ~5% stained with propidium iodide, a dye that is excluded from live cells. (B) Application of 30 μM progesterone reduced lacZ expression when B3Z cells were stimulated for 4 h by 1 μM TG alone, a combination of 1 μM TG plus 50 nM PMA (TG + PMA), immobilized anti-CD3-e antibodies, or K897 cells presenting SIIN FEKL. Fluorescence readouts from the multiwell plate reader in arbitrary units (a.u.) are presented as mean ± SD (white bars, stimulation alone; hatched bars, stimulation plus 30 μM progesterone). *Significance was determined with one-tail t tests (P < 0.0001).
Progesterone inhibits [Ca\(^{2+}\)] oscillations induced by TCR ligation. (A) [Ca\(^{2+}\)] responses from four representative B3Z cells activated by contact with SIINFEKL-presenting K897 cells illustrate that [Ca\(^{2+}\)] oscillations were reversibly inhibited by the application of 50 \(\mu\)M progesterone to the bath (bar). (B) [Ca\(^{2+}\)] oscillations in four B3Z cells activated by settling onto coverslips coated with anti-CD3-ε antibodies in the absence (a) or presence (b) of 50 \(\mu\)M progesterone.

Figure 2. Progesterone inhibits [Ca\(^{2+}\)] oscillations induced by TCR ligation. (A) [Ca\(^{2+}\)] responses from four representative B3Z cells activated by contact with SIINFEKL-presenting K897 cells illustrate that [Ca\(^{2+}\)] oscillations were reversibly inhibited by the application of 50 \(\mu\)M progesterone to the bath (bar). (B) [Ca\(^{2+}\)] oscillations in four B3Z cells activated by settling onto coverslips coated with anti-CD3-ε antibodies in the absence (a) or presence (b) of 50 \(\mu\)M progesterone.
is more potent when evaluated at the end of a 200-ms pulse (Fig. 5 C, filled triangles). The apparent increased rate of channel inactivation (Fig. 5, A and B) suggests that progesterone may preferentially bind to and block the open or inactivated Kv1.3 channel. To determine if steady-state inactivation enhances the block by progesterone, we inactivated a significant fraction of Kv1.3 channels by decreasing the holding potential from −80 to −50 mV. As shown in Fig. 6 A, progesterone (30 μM) reduced the peak Kv1.3 currents more potently when the holding potential was −50 mV (70% block; see open circle in Fig. 5 C) than when the holding potential was −80 mV (45% block), demonstrating that channel inactivation effectively enhances the block of Kv1.3 currents by progesterone. During antigen-induced oscillations of [Ca^{2+}]_{i}, Kv1.3 channels would undergo repetitive cycles of activation, inactivation, and recovery as the membrane potential fluctuates. Activation cycles can result in frequency or use-dependent inhibition if the interval between depolarizations is less than the time required for full recovery from inactivation; normally, pulse intervals of >20 s allow full recovery. In the presence of progesterone, Kv1.3 currents steadily declined during repetitive pulsing, because channel inactivation recovers 10-fold more slowly, resulting in accumulation of channels in the inactivated state (Fig. 6, B and C).

We also examined the effects of progesterone on the smaller component of Kv currents by selectively blocking the Kv1.3 component with 100 nM CTX. At this dose, we expect a residual Kv1.3 current of only 2% that in the absence of CTX. To determine if the interval between depolarizations is less than the time required for full recovery from inactivation; normally, pulse intervals of >20 s allow full recovery.
tivated human T cells with solutions containing 1 μM Ca\(^{2+}\) activated a large K\(^+\) current that was evident in voltage ramps (Fig. 8 A). Progesterone blocked K\(_{Ca}\) channels with an IC\(_{50}\) value of 113 ± 9 μM (Fig. 8 B). Thus, progesterone blocks the K\(_V\) currents more potently than the K\(_{Ca}\) currents in T cells. In contrast, the progesterone antagonist RU 486 was consistently more potent than progesterone in blocking K\(_{Ca}\) current (Fig. 8 C). These experiments demonstrate some degree of selectivity of the two components of K\(_V\) current and the K\(_{Ca}\) current for progesterone and RU 486.

Selectivity of Progesterone for K\(_V\) Channels: Progesterone Does Not Block CRAC or Chloride Channels in T Cells. The results from patch-clamp experiments suggested that progesterone, RU 486, and perhaps other steroid hormones might block several K\(_V\) channel types, albeit with rather low affinity. Therefore, we screened a panel of steroids on the K\(_V\) and K\(_{Ca}\) channels in T lymphocytes. Most of the compounds tested either had no effect or were less effective than progesterone in blocking K\(_V\) or K\(_{Ca}\) currents (Table 1). In addition, we screened several channel types, including both cloned and native channels expressed in a variety of cell lines, for block by progesterone, as summarized in Table 2. Progesterone blocks several Kv1 family members.

K\(_{Ca}\) channels hyperpolarize the membrane potential of T cells during the [Ca\(^{2+}\)] signal, effectively counteracting the depolarizing effects of Ca\(^{2+}\) influx. K\(_{Ca}\) channels are voltage-independent and highly sensitive to a rise in [Ca\(^{2+}\)], with half-activation at ~400 nM and a steep [Ca\(^{2+}\)] dependence, suggesting that at least four Ca\(^{2+}\) ions must bind to open the channel (for a review, see reference 24). Based upon biophysical characterization and its expression in T cells, it is likely that the K\(_{Ca}\) channel in T cells is encoded by the gene hKC4 (39). Intracellular dialysis of B3Z or ac-

### Selectivity of Progesterone for K\(_V\) Channels: Progesterone Does Not Block CRAC or Chloride Channels in T Cells.

- **Figure 7.** Progesterone blocks CTX-resistant K\(_V\) channels. Pipette solution as in Fig. 5. (A) C current responses of the total K\(_V\) current (●) and the CTX-resistant current (■) to repetitive voltage pulses from −80 to +30 mV separated by 1 s. At this rate of pulsing, K\(_{V,1.3}\) channels undergo cumulative inactivation, as shown by normalized peak current amplitudes in the absence of CTX (■). With 100 nM CTX present (●), the remaining current does not decline during repetitive pulsing. (B) Progesterone or RU 486 (50 μM) blocks the K\(_V\) component. (C) Progesterone blocks the CTX-resistant component of K\(_V\) current more than RU 486. 100 nM CTX was preapplied in Ringer in order to block K\(_{V,1.3}\) channels.

- **Figure 8.** Concentration-dependent inhibition of K\(_{Ca}\) current in B3Z cells by progesterone. (A) K\(_{Ca}\) currents were activated by dialyzing the cell with a solution containing (in mM) 140 K\(^+\) aspartate, 2 Mgly, 7.8 CaCl\(_2\), 10 EGTA, 5 Hepes (pH 7.2). The nominal free Ca\(^{2+}\) concentration of this solution was 1 μM, assuming a dissociation constant for EGTA and Ca\(^{2+}\) of 10\(^{-7}\) at pH 7.2. Ca\(^{2+}\)-activated K\(^+\) currents were evoked by voltage ramps of 200-ms duration from −120 to +50 mV every 30 s. Application of progesterone at different concentrations (indicated at the right of each trace) inhibited the K\(_{Ca}\) current. (B) Concentration-response curve for progesterone block of K\(_{Ca}\) currents (●). The slope conductance between −100 and −60 mV was used as a measure of the K\(_{Ca}\) conductance to avoid contamination by K\(_V\) currents. Data were normalized to the conductance measured in the absence of progesterone and presented as mean ± SD. The line represents the fit to a Hill equation with IC\(_{50}\) 113 μM and n = 1.2. Block of K\(_{Ca}\) channels by 60 μM RU 486 (□) is shown for comparison. (C) Comparison of progesterone and RU 486. Slope conductance values at −80 mV illustrate activation and block of the K\(_{Ca}\) current by RU 486 and progesterone, each applied at 60 μM.
including Kv1.3 expressed in lymphocytes as the predominant Kv current, as well as Kv3.1 expressed in the brain and in certain subsets of mouse thymocytes. In contrast, progesterone had very little effect on a cloned voltage-gated Na\(^+\) channel found in skeletal muscle, or on a strongly inward rectifying K\(^+\) channel found in RBL cells. We conclude that progesterone is a broad spectrum, low-affinity K\(^+\) channel blocker.

In further experiments on Jurkat T lymphocytes, we evaluated effects of progesterone on CRAC and Cl\(^-\) channels to determine if modulation of these channel types might contribute to the inhibition of Ca\(^{2+}\) signaling. During whole-cell patch-clamp recordings, intracellular dialysis with heavily buffered low-Ca\(^{2+}\) solutions passively depleted the intracellular Ca\(^{2+}\) stores and activated CRAC channels (Fig. 9 A). After maximal activation, 30 \(\mu\)M progesterone (Fig. 9 B, application bar) had no effect on the amplitude or the current-voltage characteristics (n = 6 cells). This experiment rules out direct CRAC channel block as a possible contributor to the inhibition of Ca\(^{2+}\) signaling by progesterone; instead, it appears that progesterone blocks Ca\(^{2+}\) signaling by inhibiting K\(^+\) channels, indirectly reducing the driving force for Ca\(^{2+}\) entry. Cl\(^-\) channels have also been implicated in lymphocyte signaling mechanisms by helping to maintain E\(_m\) during T cell activation (20, 21). However, superfusion of B3Z or Jurkat cells with 50 \(\mu\)M progesterone did not affect the amplitude or the current-voltage characteristics of Cl\(^-\) currents induced by cell swelling (Fig. 9, C and D).

### Discussion

In this report, we demonstrate by patch-clamp measurement that progesterone directly blocks Kv and KCa channels, but not Ca\(^{2+}\) or Cl\(^-\) channels in T lymphocytes. Furthermore, we show that K\(^+\) channel blockade is associated with membrane depolarization, inhibition of Ca\(^{2+}\) signaling, and a reduction of NF-AT-driven gene expression. Since NF-AT links activation of the TCR to IL-2 production, interruption of these signals would inhibit production of the major proliferative cytokine for T cells. We propose that K\(^+\) channel blockade provides a mechanism contributing to the immunosuppressive effects of progesterone.

The rapid onset and reversibility of Kv channel block by progesterone is incompatible with changes in mRNA or protein synthesis, suggesting that these effects are not mediated by the classical steroid receptor pathway (40). The progesterone antagonist RU 486 is nearly as potent as progesterone in blocking both Kv and gene expression, also implicating a nongenomic action of progesterone.

### Table 1. Percent Inhibition of Kv and KCa Channels by Steroids (60 \(\mu\)M)

<table>
<thead>
<tr>
<th>Progesterone</th>
<th>RU 486</th>
<th>Estradiol</th>
<th>Testosterone</th>
<th>Cortisol</th>
<th>DHEA</th>
<th>OHP</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv (77 \pm 8)</td>
<td>61 (\pm 15)</td>
<td>24 (\pm 7)</td>
<td>17 (\pm 12)</td>
<td>13 (\pm 4)</td>
<td>10 (\pm 6)</td>
<td>12 (\pm 6)</td>
<td>12 (\pm 13)</td>
</tr>
<tr>
<td>KCa (34 \pm 11)</td>
<td>55 (\pm 12)</td>
<td>8 (\pm 10)</td>
<td>4 (\pm 7)</td>
<td>0.0 (\pm 0.1)</td>
<td>0.4 (\pm 0.7)</td>
<td>2 (\pm 4)</td>
<td>6 (\pm 7)</td>
</tr>
</tbody>
</table>

Currents through Kv channels in human T cells were evoked by depolarizing pulses to +30 mV, as in Fig. 5. KCa channels in activated human T cells were opened by 1 \(\mu\)M [Ca\(^{2+}\)], with currents measured during voltage-ramp stimuli as in Fig. 8. Data are presented as mean ± SD, with at least three separate experiments for each compound tested at 60 \(\mu\)M, and represent the percent inhibition of peak Kv currents or slope conductances of KCa currents near −80 mV. DHEA, Deydroepiandrosterone; OHP, 17α-hydroxy-4-pregnene-3,20-dione.
Progesterone blocks voltage-gated Ca\(^{2+}\) channels in smooth muscle cells and a variety of K\(^{+}\) channels in MDCK cells and hepatocytes (43–45). Several transmitter-activated channels are also suppressed by progesterone in the micromolar concentration range (46–49). In contrast to its effects on somatic cells, progesterone activates Ca\(^{2+}\) influx in sperm (50, 51). We found no evidence for progesterone-induced Ca\(^{2+}\) influx in T cells.

Our data provide the first evidence that an endogenous hormone may act as an immunosuppressant by blocking K\(^{+}\) channels. Inhibition of K\(^{+}\) channels has been shown to reduce IL-2 production and T cell activation in vitro (22, 23, 52). Moreover, recent studies demonstrated that the peptide scorpion toxin margatoxin, a specific blocker of Kv1.3 channels, inhibits delayed-type hypersensitivity reaction and reduces response to allogeneic challenge in vivo (25). The depolarization and reduction of the driving force for Ca\(^{2+}\) entry resulting from K\(^{+}\) channel inhibition are sufficient to account for the reduction of Ca\(^{2+}\) signals and NF-AT–driven gene expression. CRAC channels are inwardly rectifying, and a modest depolarization can reduce Ca\(^{2+}\) entry significantly, reducing the rise in [Ca\(^{2+}\)]\(_i\) below the threshold for gene expression (for a review, see reference 24). At high concentrations, progesterone reduces Ca\(^{2+}\) signaling and gene expression almost to control levels, below a plateau level seen with 100 nM CTX (21, 53, and data not shown). Progesterone, although acting with low affinity, may reduce Ca\(^{2+}\) signaling and gene expression to a greater extent than CTX because progesterone also inhibits CTX-resistant Kv channels. The block of K\(^{+}\) channels by progesterone or RU 486 can also account for previous results showing that progesterone or RU 486 inhibits activation of human T cells in vitro (8, 9), as well as the reduction of the number of CD3\(^{+}\) cells in the placenta compared with maternal blood (11, 54).

During pregnancy, immunoregulatory mechanisms must operate locally at the placental interface and be readily reversible to preserve the systemic immune competence of the mother. Several mechanisms involving progesterone may contribute to fetal–maternal protection, including altered expression of MHC class I proteins in fetal tissue, altered T cell subsets, or elaboration of immunosuppressive factors (2). Biochemical measurements have estimated progesterone concentrations to be 20 \(\mu\)M within the placenta (34, 35); concentrations in the vicinity of trophoblasts producing progesterone must be even higher. Average progesterone levels found in the placenta would be sufficient to block lymphocyte K\(^{+}\) channels and thereby mediate a highly localized and reversible immunosuppression without compromising the maternal immune system. The affinity of progesterone for K\(^{+}\) channels ensures that this mechanism would only be effective in the region of potential contact between allogeneic cells, where progesterone is present at high concentrations.
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