PGE₂ suppresses mitogen-induced Ca²⁺ mobilization in T cells

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Choudhry, Mashkoor A., Philip E. Hockberger, and Mohammed M. Sayeed. PGE₂ suppresses mitogen-induced Ca²⁺ mobilization in T cells. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1741–R1748, 1999.—PGE₂-mediated suppression of T cell proliferation during sepsis could result from altered Ca²⁺ signaling. The present study evaluated the effects of PGE₂ on Ca²⁺ release from intracellular stores and its influx through the plasma membrane in splenic T cells from Sprague-Dawley rats. Intracellular Ca²⁺ concentration ([Ca²⁺]i) responses in individual T cells were assessed using the Ca²⁺ imaging technique, and the release of Ca²⁺ from intracellular stores and Ca²⁺ influx were spectrofluorometrically quantified in T cell suspensions. Under unstimulated conditions, nearly 85% of T cells exhibited [Ca²⁺]i ≤ 50 nM. After stimulation with concanavalin A (Con A), an increase in [Ca²⁺]i was recorded in ~60% of the cells. The pretreatment of T cells with PGE₂ had no apparent effect on [Ca²⁺]i in resting cells; it significantly suppressed the Con A-induced increase in [Ca²⁺]i in all of the Con A-responsive cells. Ca²⁺ release from the intracellular stores contributed to the early spike in [Ca²⁺]i, and the late phase of elevation in [Ca²⁺]i was dependent on Ca²⁺ influx through the plasma membrane. Our data suggest that PGE₂ causes an overall suppression of the Con A-induced [Ca²⁺]i elevation in T cells via inhibiting both Ca²⁺ influx and its release from the intracellular stores.

Concanavalin A; T lymphocytes; calcium ion signaling; intracellular calcium ion release; adenosine 3,5-cyclic monophosphate; prostaglandin E₂

T cell activation and interleukin (IL)-2 production are essential for appropriate functioning of the immune system (1, 2, 35). The activation of T cells is effected primarily via stimulation of T cell antigen receptor (TCR). Two of the TCR polypeptide chains forming a heterodimer are important in recognizing the antigen; the others, collectively called CD3, are involved in receptor assembly and signal transmission (2, 36). The stimulation of TCR, in vivo, results from its interaction with antigen-presenting cells (1, 2, 35). In vitro, TCR stimulation could be achieved with lectins or specific antibodies directed against the CD3 complex (1, 2, 36) and gives rise to a series of intracellular responses eventually leading to T cell IL-2 production (1, 2, 4, 12, 35, 36). The intracellular responses include the activation of protein tyrosine kinases and phospholipase C-γ (1, 2, 4) with subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol 1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol. The IP₃-mediated release of Ca²⁺ from intracellular stores is followed by Ca²⁺ influx through the plasma membrane causing a sustained elevation in intracellular Ca²⁺ concentration ([Ca²⁺]i); see Refs. 1, 2, 4, 5, 12, 14, 37). The increase in [Ca²⁺]i sustained for several hours is considered critical for T cell activation and IL-2 production (2, 4, 5, 21, 37).

An increased production and release of PGE₂ after burn and septic injuries has been correlated with a decrease in T cell IL-2 production and proliferation (8, 18, 20, 33). The role of PGE₂ in the suppression of T cell IL-2 production and proliferation is borne out by direct effects of PGE₂ on T cells (6, 7, 24, 26, 28, 34). In previous studies, we showed that PGE₂-mediated suppression of T cell functions is associated with an attenuation in T cell Ca²⁺ signaling as assessed in T cell suspensions (8, 10).

In the present study, we evaluated the effects of PGE₂ on [Ca²⁺]i responses in individual T cells using the Ca²⁺ imaging technique. These assessments allowed us to ascertain variability in the [Ca²⁺]i responses within the T cell population. We examined also the effects of PGE₂ on release of Ca²⁺ from intracellular stores and its influx through the plasma membrane. Additionally, we ascertained the effect of intracellular cAMP, the second messenger generated in the action of PGE₂, in the modulation of T cell [Ca²⁺]i (3, 17, 23, 34).

EXPERIMENTAL PROCEDURES

Animals and reagents. Male Sprague-Dawley rats (200–225 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). PGE₂, dibutyryl-cAMP (DBcAMP), EGTA, and concanavalin A (Con A) were purchased from Sigma Chemical (St. Louis, MO). Fura 2-AM was purchased from Molecular Probes (Eugene, Oregon). Thapsigargin was obtained from Calbiochem-Novabiochem International (San Diego, CA). Ficol-Paque and nylon wool fiber were obtained, respectively, from Pharmacia Sweden and Polysciences (Warrington, PA). All other cell culture reagents were purchased from GIBCO-BRL (Grand Island, NY).

T cell preparation. Anesthetized rats were killed, and their spleens were removed. Spleens were minced to dissociate lymphocytes into single cells, and suspensions of single cells were prepared in Hanks’ balanced salt solution (HBSS). Red blood cells present in these cell suspensions were removed using Ficol-Paque. For the enrichment of T cells, splenocytes were passed through a nylon wool column preequilibrated with HBSS containing 10 mM HEPES, 5% FCS, and 50 µg/ml gentamicin. After 45–60 min of incubation of the columns with cells at 37°C, T cells were obtained by eluting the columns with 20–25 ml of warm HBSS. Details of the T cell preparation procedure have been reported previously (8, 10, 11).
Measurements of single cell [Ca\(^{2+}\)]\(_i\) using Ca\(^{2+}\) imaging. T cell suspensions were loaded with 10 µM fura 2-AM for 1 h at room temperature as described earlier (8, 10). Approximately 100 µl of the cell suspension were placed on a coverslip and examined under a ×40 objective of an inverted microscope (Nikon). The cells were exposed to alternating 340- and 380-nm excitation wavelengths, and emission of fura 2 was collected through a 505-nm band-pass filter. Images were obtained using a cooled-CCD-Camera (Sensys; Photometrics) and image acquisition system (Universal Imaging). Images were corrected for background fluorescence, separated into ratios, and analyzed using Metafluor software (Universal Imaging; see Ref. 25). [Ca\(^{2+}\)]\(_i\) was estimated by calibrating the imaging system with mixtures of solutions of known Ca\(^{2+}\) and fura 2 concentrations (27).

Fluorometric measurements of [Ca\(^{2+}\)]\(_i\) in T cell suspension. Fura 2-loaded cells were transferred to a cuvette, and fluorescence signals were recorded using a Hitachi Spectrofluorometer (model F-2000) at excitation wavelengths of 340 and 380 nm and emission at 510 nm. Details of the fluorometric techniques for determining [Ca\(^{2+}\)]\(_i\) have been described elsewhere (8, 10). EGTA (3 mM) was used in some experiments to lower extracellular [Ca\(^{2+}\)]\(_i\) to 150 nM. IP\(_3\)-mediated Ca\(^{2+}\) release in T cells was carried out after permeabilizing the cells with saponin (150 µg/ml), as described earlier (15). [Ca\(^{2+}\)]\(_i\) were recorded after calibration of the fluorescent signals using Ca\(^{2+}\) standard solutions (27). Results were digitized and imported into a statistical analysis program (Statistical package for Social Sciences Software Program, version 2.0; SigmaStat, Chicago, IL) for quantitative analyses. Normalized [Ca\(^{2+}\)]\(_i\) responses shown in some figures were calculated in the following two steps: 1) the lowest basal [Ca\(^{2+}\)]\(_i\) was subtracted from all of the [Ca\(^{2+}\)]\(_i\) values, and 2) the resulting [Ca\(^{2+}\)]\(_i\) values were divided by maximum [Ca\(^{2+}\)]\(_i\) value. This allowed us to hold the maximum [Ca\(^{2+}\)]\(_i\) equal to one and then express remaining [Ca\(^{2+}\)]\(_i\) values as fractions of one. Integrated [Ca\(^{2+}\)]\(_i\) were determined by calculating the area under the [Ca\(^{2+}\)]\(_i\) response curve; the integrated [Ca\(^{2+}\)]\(_i\) values were pooled separately for control and experimental groups and were presented as means ± SE. Unless mentioned, integrated [Ca\(^{2+}\)]\(_i\) in T cells were calculated over the period of 300 s starting from the time of concanavalin A (Con A) stimulation. In the present study, we determined the effects of various Con A concentrations on T cell [Ca\(^{2+}\)]\(_i\) elevation and found T cell stimulation to be maximal at the 100-µg/ml concentration. Therefore, we employed the 100-µg/ml concentration in this series of experiments. We used 10 µM PGE\(_2\) in all PGE\(_2\)-related experiments.

The data, wherever applicable, are presented means ± SE and were analyzed using ANOVA (Statistical package for Social Sciences Software Program, version 2.0; SigmaStat). A P ≤ 0.05 between the two groups was considered as statistically significant. The experiments described here were conducted in adherence to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

RESULTS

[Ca\(^{2+}\)]\(_i\) images in individual T cells before and ~150 s after their stimulation with the mitogen (Con A) are shown in Fig. 1. In unstimulated conditions, almost all cells appeared blue, a color corresponding to 20–60 nM [Ca\(^{2+}\)]\(_i\) as shown on the pseudocolor scale bar (Fig. 1A).
Similarly, unstimulated T cells incubated with PGE$_2$ also exhibited a color that corresponds to 20–60 nM [Ca$^{2+}$] (Fig. 1C). Addition of Con A caused an increase in [Ca$^{2+}$]$_i$ to different levels in different cells. Such [Ca$^{2+}$]$_i$ increases ranged from 90 to 200 nM (Fig. 1B). The stimulation of cells with Con A after PGE$_2$ treatment failed to cause an increase in [Ca$^{2+}$]$_i$ (Fig. 1D). As can be seen from Fig. 2A, 85 ± 5.2% of unstimulated T cells had [Ca$^{2+}$]$_i$ ≤50 nM, which is likely the basal [Ca$^{2+}$]. After stimulation with Con A, 40 ± 8.7% of the cells maintained [Ca$^{2+}$]$_i$ ≤50 nM, suggesting that the remaining ~60% of the basal state cells were activated by Con A to attain [Ca$^{2+}$]$_i$ ≥50 nM. Con A apparently caused increases in the number of cells with [Ca$^{2+}$]$_i$ at 100, 150, and 200 nM. PGE$_2$ did not have a demonstrable effect on [Ca$^{2+}$]$_i$ distribution before stimulation of cells with Con A (Fig. 2B). In the PGE$_2$-treated group, [Ca$^{2+}$]$_i$ was ≤50 nM in 88 ± 0.9% of the unstimulated cells and 72 ± 9% of Con A-stimulated cells, suggesting that Con A caused increases in [Ca$^{2+}$]$_i$ in only ~16% of the PGE$_2$-treated cells. This level of stimulation by Con A is apparently less than that observed in the unstimulated T cells.

The loss of [Ca$^{2+}$]$_i$, response to mitogen in PGE$_2$-treated T cells could result from a decrease either in Ca$^{2+}$ release from intracellular stores, from a decrease in Ca$^{2+}$ influx, or both. We first determined the kinetics and relative contributions of these two mechanisms in T cell suspensions without added PGE$_2$. Without the exposure of cells to EGTA, the stimulation by Con A resulted in a rapid increase in [Ca$^{2+}$]$_i$ followed by a slow recovery (over an ~200-s duration) that was incomplete and then by a second slowly rising phase of the [Ca$^{2+}$]$_i$ (Fig. 3A). In the presence of 3 mM EGTA, the kinetics and the magnitude of the initial rise in [Ca$^{2+}$]$_i$ were not different from those observed in the absence of EGTA. However, the subsequent decline in [Ca$^{2+}$]$_i$ was more rapid and was not followed by the second rise in [Ca$^{2+}$]$_i$ (Fig. 3B). The second rise in [Ca$^{2+}$]$_i$ in the absence of EGTA was presumably due to Ca$^{2+}$ influx (Fig. 3C). We compared the areas under the [Ca$^{2+}$]$_i$ curves to estimate integrated [Ca$^{2+}$]$_i$ responses (over the period of 300 s starting from time of Con A addition to the cells) in the absence and presence of EGTA. Con A caused an increase in integrated [Ca$^{2+}$]$_i$ (3.51 ± 2.22 × 10$^4$ nM/s, mean ± SE from 8 different animals) in the absence of EGTA that was significantly higher (P < 0.05) than that obtained in the presence of EGTA (1.07 ± 0.05 × 10$^4$ nM/s). The difference between the two [Ca$^{2+}$]$_i$ responses (~2.44 × 10$^4$ nM/s) probably represented the magnitude of Ca$^{2+}$ influx from the extracellular space in cells stimulated with Con A. The integrated responses indicate that, during the initial 300 s of Con A stimulation of control T cells, ~40% of the total [Ca$^{2+}$]$_i$ originated from the intracellular stores and ~60% originated from the extracellular pool. These data showed also that the Con A-mediated initial rapid rise in [Ca$^{2+}$]$_i$ in control T cells was primarily due to release of Ca$^{2+}$ from the intracellular stores and that Ca$^{2+}$ influx was initiated after peak [Ca$^{2+}$]$_i$ was achieved. This finding provided us with the rationale for examining the effect of PGE$_2$ on Ca$^{2+}$ influx by applying it to T cells at the time of peak [Ca$^{2+}$]$_i$, response to the mitogen. PGE$_2$ addition at the peak [Ca$^{2+}$]$_i$ (attained at ~100 s after Con A stimulation) increased the rate of the
subsequent decline in \([\text{Ca}^{2+}]_i\) relative to that observed in the absence of PGE2 (Fig. 4). Furthermore, we found that \([\text{Ca}^{2+}]_i\) elevation in the presence of PGE2 returned to the basal level in ~300 s, whereas, in the presence of 3 mM EGTA, this took ~200 s. Because the rate of \([\text{Ca}^{2+}]_i\) decline with PGE2 was still slower than that which occurred in the presence of EGTA, it can be surmised that PGE2 causes a net decrease in cytosolic \([\text{Ca}^{2+}]_i\) accumulation by decreasing \([\text{Ca}^{2+}]_i\) influx.

We also examined the effects of the second messenger of PGE2, cAMP, by applying DBcAMP to T cells at the peak \([\text{Ca}^{2+}]_i\) response to Con A. Like PGE2, DBcAMP augmented the rate of decline of \([\text{Ca}^{2+}]_i\). The \([\text{Ca}^{2+}]_i\) decline to basal level in the presence of DBcAMP occurred over a time period comparable to that observed with PGE2 (~540 s after Con A). To compare the effects of PGE2 with that of DBcAMP on \([\text{Ca}^{2+}]_i\) influx, we integrated the area under the \([\text{Ca}^{2+}]_i\) response curve from 340 s after Con A addition to the time of return of \([\text{Ca}^{2+}]_i\) to near basal levels, a time interval during which the \([\text{Ca}^{2+}]_i\) elevation was presumably due to influx alone (Fig. 3C). The average integrated \([\text{Ca}^{2+}]_i\) response values obtained from T cells of eight different
animals are shown in Fig. 5. In the absence of PGE2 or DBcAMP, the integrated [Ca^{2+}]_{i} in T cells after their stimulation with Con A was found to be \(2.15 \pm 0.35 \times 10^{4}\) nM/s. This [Ca^{2+}]_{i} response was significantly \((P < 0.01)\) suppressed when the cells were treated with PGE2 or DBcAMP after Con A stimulation.

A more definitive PGE2 effect on Ca^{2+} influx is indicated by data in Fig. 6. Thapsigargin, which presumably inhibited Ca^{2+} uptake into the intracellular reservoir and caused depletion of the reservoir, allowed for the monitoring of Ca^{2+} influx after CaCl2 was added to the extracellular compartment. Figure 6 shows a cessation of the Ca^{2+} influx with the reintroduction of additional quantities of EGTA. PGE2 addition after CaCl2 was evidently effective in attenuating the influx of Ca^{2+}.

To assess the potential effect of PGE2 on intracellular Ca^{2+} release, 10 µM PGE2 was first added to T cells followed by the addition of 3 mM EGTA 100 s later. Con A was then added when a stable T cell [Ca^{2+}]_{i} was established, \(\sim 100\) s after EGTA. T cell [Ca^{2+}]_{i} changes after these additions are shown in Fig. 7, A and B. In these experiments, we noticed a higher basal [Ca^{2+}]_{i} in T cells (Fig. 7, A and B). The [Ca^{2+}]_{i} was reduced to \(\sim 70\) nM after adding 3 mM EGTA. As shown in Fig. 7, Con A-induced Ca^{2+} release in the presence of EGTA and PGE2 \((0.94 \pm 0.16 \times 10^{4}\) nM/s, mean integrated [Ca^{2+}]_{i} \pm SE values obtained from 6 different animals) was not significantly different from that observed in T cells exposed to EGTA alone \((0.95 \pm 0.15 \times 10^{4}\) nM/s, \((A)\) and B) or inositol 1,4,5-trisphosphate (IP3; C and D). For IP3-mediated release, T cells were permeabilized by incubating with saponin (150 µg/ml) for \(30-45\) min at room temperature. Traces are typical of results obtained from cells of 6 different animals.
we have now examined Ca$^{2+}$ absence of PGE$_2$ induced an elevation in [Ca$^{2+}$]i in T cells with or without pretreatment with PGE$_2$. Cells were first permeabilized with saponin (150 µg/ml) for ~40–60 min and then were stimulated with IP$_3$ after their exposure to PGE$_2$. After establishing a stable [Ca$^{2+}$]i elevation in T cells preincubated with PGE$_2$, we showed a suppression in the initial [Ca$^{2+}$]i rise in T cells preincubated with PGE$_2$ for 2 h. As we found in this study, the initial rise is primarily due to Ca$^{2+}$ release from intracellular stores. A suppression in the initial Ca$^{2+}$ rise in T cells incubated with PGE$_2$ for 2 h could also result from a decrease in Ca$^{2+}$ release from intracellular stores. To elucidate the effects of long-term exposure (~2 h pre-treatment) of PGE$_2$ on T cell intracellular Ca$^{2+}$ release, we have now examined Ca$^{2+}$ release in the presence of EGTA (Fig. 8). We found that Con A-mediated [Ca$^{2+}$]i elevation in the presence of 3 mM EGTA was significantly (P < 0.01) suppressed in the T cells preincubated with PGE$_2$ (0.81 ± 0.05 × 10$^4$ nM/s, mean integrated [Ca$^{2+}$]i ± SE values obtained from 6 different animals) compared with T cells incubated without PGE$_2$ (1.21 ± 0.08 × 10$^4$ nM/s, mean integrated [Ca$^{2+}$]i ± SE values obtained from 6 different animals). These data indicated that a prolonged incubation of T cells with PGE$_2$ led to a decrease in both Ca$^{2+}$ influx and release from intracellular stores.

**DISCUSSION**

The role of PGE$_2$ as an immunosuppressant has been amply established (8, 18, 20, 33). Specifically, PGE$_2$ is known to suppress T cell IL-2 production and proliferation (6, 7, 8, 10, 18, 20, 23, 24, 34). A number of studies have shown burn/sepsis injury-related alterations in T cell IL-2 transcriptional regulation (6, 24, 28). Previous studies from our laboratory have shown an impairment in T cell early signaling events, including intracellular Ca$^{2+}$ mobilization in sepsis (8, 9). We showed also that the treatment of septic animals with the PGE$_2$ blocker indomethacin prevented the sepsis-related suppression in T cell Ca$^{2+}$ signaling and the proliferative responses (8, 10). Similar changes in Ca$^{2+}$ signaling were implicated in trauma-induced T cell functional disturbances (20). On the other hand, Faist et al. (16) suggested a primary role of protein kinase C (PKC) in trauma-related suppression of T cell proliferation. The role of PKC in PGE$_2$-mediated T cell proliferative disturbances was also supported by the studies of Chouaib et al. (7). They suggested that, although PGE$_2$ affected both Ca$^{2+}$ mobilization and PKC activation, the restitution of Ca$^{2+}$ signal partially restored T cell proliferation, whereas PKC activation via tetradecanoylphorbol 13-acetate (TPA) completely restored the T cell response. This is understandable because, although the activation of some PKC isoforms is dependent on Ca$^{2+}$, certain other isoforms are independent of Ca$^{2+}$. TPA stimulation would be expected to have near-maximal stimulation of both Ca$^{2+}$-dependent and -independent PKC, leading to an abrogation of the PGE$_2$-mediated suppression. Stimulation of T cells with mitogen or antigen results in a cascade of intracellular events, including elevation in [Ca$^{2+}$]i and PKC activation (1, 2, 4, 33). Recent studies have suggested that the sustained elevation in [Ca$^{2+}$]i for several hours is critical for T cell IL-2 production and subsequent proliferation (2, 4, 21, 37).

Recent studies have shown that anti-CD3 or mitogen stimulation of T cells induced Ca$^{2+}$ signals of diverse magnitudes in a population of T cells and that only 20–30% of the cells responded to such stimuli (19). These findings would imply that effects of PGE$_2$ on T cell Ca$^{2+}$ signaling are likely mediated through the action of PKC on the 20–30% anti-CD3 or mitogen-sensitive cell population. Our previous assessments of Ca$^{2+}$ signaling in T cell suspensions had also indicated variations in responsiveness within the T cell population. In the present study, Ca$^{2+}$ imaging in individual T cells indicated that the addition of Con A to control T cells elevated [Ca$^{2+}$]i in ~50–60% of the cells present in the microscopic field. The difference in our

![Fig. 8. Representative traces from spectrophotofluorometric assessments showing effects of PGE$_2$ on Con-A-mediated [Ca$^{2+}$]i elevation in T cells in the presence of 3 mM EGTA. T cells were incubated with and without PGE$_2$ (10 µM) for 2 h before [Ca$^{2+}$]i measurements at 37°C. Traces are typical of results obtained from cells of 6 different animals.](http://ajpregu.physiology.org/ by 10.220.33.6 on June 28, 2017)
data were pooled from five different animals (Fig. 2). However, this decrease was not demonstrable when the decrease in T cell Ca\(^{2+}\) cells incubated in the absence of PGE\(_2\) (Fig. 1A). This number of recent studies suggested that capacitive depletion of cells represents a store-dependent influx pathway. Our present study demonstrated a decrease in Ca\(^{2+}\) stores to release Ca\(^{2+}\) from the intracellular reservoir. Our present study demonstrated a decrease in Ca\(^{2+}\) release from intracellular stores when T cells were preincubated with PGE\(_2\) for 2 h, although the treatment of T cells with PGE\(_2\) immediately before their stimulation with Con A or at peak [Ca\(^{2+}\)]\(_i\) response to Con A failed to affect the capability of intracellular stores to release Ca\(^{2+}\) or the sensitivity of the release mechanism to IP\(_3\). These findings suggest that, although prolonged exposure of T cells to PGE\(_2\) (2 h) before cross-linking of the TCR with Con A could attenuate Con-A-mediated formation of IP\(_3\), such IP\(_3\) generation may not be compromised immediately after PGE\(_2\) application to T cells. We speculate that the trigger for Ca\(^{2+}\) release from intracellular stores may be affected only after the prolonged PGE\(_2\) exposure.

Previous studies have shown that, although Ca\(^{2+}\) influx is important in the maintenance of the sustained elevation of T cell [Ca\(^{2+}\)]\(_i\) (2, 5, 14, 21, 37), all of the Ca\(^{2+}\) entering the cell may not contribute to the [Ca\(^{2+}\)]\(_i\) elevation. Some of the Ca\(^{2+}\) are presumably either extruded via a Ca\(^{2+}\) efflux mechanism or pumped into the intracellular Ca\(^{2+}\) stores (4, 14, 29). An alteration in any of these mechanisms could also affect the sustained Ca\(^{2+}\) elevation. As pointed out in results, the influx of Ca\(^{2+}\) was observed (Fig. 6) in T cell depleted of the intracellular Ca\(^{2+}\) store. The influx of Ca\(^{2+}\) in store-depleted cells represents a store-dependent influx pathway referred to as the "capacitive Ca\(^{2+}\) entry" (31, 32). A number of recent studies suggested that capacitive Ca\(^{2+}\) entry in various cell systems, including the T cells, is activated by the depletion of the Ca\(^{2+}\) store and is terminated by subsequent refilling (13, 29, 30, 38). We evaluated the effects of PGE\(_2\) on Ca\(^{2+}\) entry in T cells after their exposure to thapsigargin in the presence of 3 mM EGTA. In the presence of EGTA, thapsigargin allowed for the depletion of the Ca\(^{2+}\) stores and prevented their refilling. The results in Fig. 6 showing the elevation in [Ca\(^{2+}\)]\(_i\) after restitution of Ca\(^{2+}\) in the extracellular medium suggest capacitive Ca\(^{2+}\) entry in rat T cells. These studies are consistent with earlier observations in human transformed Jurkat T cells and other cell systems (13, 31). The elevation in T cell [Ca\(^{2+}\)] in the presence of thapsigargin was presumably due to entry from extracellular medium, as reintroduction of EGTA led to return of [Ca\(^{2+}\)]\(_i\), to basal levels. Similar to EGTA, addition of PGE\(_2\) also produced a marked suppression in [Ca\(^{2+}\)]\(_i\) elevation. These results supported the hypothesis that the PGE\(_2\)-mediated decrease in sustained elevation could also result from an attenuation in a capacitive Ca\(^{2+}\) entry. The decrease in [Ca\(^{2+}\)]\(_i\) elevation due to a decrease in Ca\(^{2+}\) influx and its release from intracellular stores is likely a major contributor to PGE\(_2\)-mediated suppression of T cell responses.

Technical assistance by Dr. Z. Ahmed and L. Amato is acknowledged.

This study was supported by National Institute of General Medical Sciences Grants ROI GM 53235 and ROI GM 56865.

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Received 2 March 1999; accepted in final form 26 July 1999.

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