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Superantigen-Induced Steroid Resistance Depends on Activation of Phospholipase C\(\beta\)2

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The glucocorticoid receptor is present in a TCR-associated complex, which includes the Src family tyrosine kinase Lck. Glucocorticoids rapidly dissociate this complex, resulting in the inhibition of canonical Lck-phospholipase C (PLC)\(\gamma\)-dependent TCR signaling. The relative importance of this nongenomic role for the glucocorticoid receptor compared with its direct transcriptional effects is not known. Superantigens induce a state of steroid resistance in activated T cells. It was reported that, in addition to canonical Lck-PLC\(\gamma\) signaling, superantigens can activate a noncanonical G protein–PLC\(\beta\)-dependent signaling pathway. In this study, we show that staphylococcal enterotoxin B activates a Geq and PLC\(\beta\)2-dependent pathway in human T cells. We find that this pathway bypasses the need for canonical Lck-PLC\(\gamma\) signaling in T cell activation and renders superantigen-stimulated T cells insensitive to glucocorticoids in vitro. We show that the PLC\(\beta\) inhibitor U-73122 sensitizes staphylococcal enterotoxin B–treated mice to dexamethasone in vivo. In conclusion, we find that effects of glucocorticoids on TCR-induced T cell proliferation are mainly nongenomic and can be bypassed by the activation of an Lck-independent signaling pathway. The Journal of Immunology, 2013, 190: 6589–6595.

Glucocorticoids bind the glucocorticoid receptor (GR), which subsequently dimerizes and translocates from the cytosol to the nucleus. The GR can bind to specific DNA sequences and alter the transcriptional activity of a wide range of genes, many of which play a role in the immune system. However, the GR also can affect cell signaling in its DNA-unbound state (so-called “nongenomic” or “nontranscriptional effects”) (1). We found previously that the GR is present in a TCR-associated complex that contains the Src-like tyrosine kinase Lck (2). Glucocorticoids rapidly dissociate this complex, leading to the inactivation of Lck and downstream signaling pathways (2, 3). Lck mediates canonical TCR signaling via activation of ZAP-70, phosphorylation of LAT, and the assembly of a multiprotein complex. A key component of this complex is phospholipase C (PLC)\(\gamma\), which converts phosphatidylinositol 4,5-bisphosphate to inositol 3,4,5-triphosphate and diacylglycerol. Generation of inositol 3,4,5-triphosphate activates Ca\(\text{2+}\) signaling and leads to the nuclear translocation of NFAT, whereas diacylglycerol activates PKC and RAS-MAPK signaling (see Fig. 1A) (4). Glucocorticoids inhibit T cell activation, but the functional relevance of loss of TCR-associated GR and the resulting inactivation of Lck versus transcriptionally mediated effects of the GR is not known. If the dissociation and inactivation of Lck are an important part of the mechanism of action of steroids, this predicts that signals that bypass Lck render T cells relatively insensitive to steroids. Superantigens activate T cells by directly cross-linking MHC class II to the TCR as unprocessed proteins. Therefore, superantigens bypass the specificity of conventional Ag recognition and can activate up to 20% of the total number of T cells (5). The massive activation of the adaptive immune system that ensues helps the pathogen to evade an appropriate immune response. The release of cytokines, such as IL-1, TNF-\(\alpha\), and IFN-\(\gamma\), causes the signs and symptoms of toxic shock syndrome (TSS) (6). Superantigen signaling is an excellent model to address the question of the relevance of glucocorticoid-mediated inhibition of upstream T cell signaling. Superantigens were reported to cause steroid resistance in T cells (7). More importantly, it was shown that superantigens can activate a noncanonical G protein–PLC\(\beta\) signaling pathway in addition to canonical Lck-PLC\(\gamma\) signaling (8). If steroid-mediated inhibition of T cell proliferation depends mainly on nongenomic inhibition of canonical T cell signaling, this predicts that superantigen-mediated glucocorticoid resistance depends on PLC\(\beta\) signaling.

In this study, we find that superantigen-mediated steroid resistance depends on Geq-mediated activation of PLC\(\beta\)2, which bypasses dependence on Lck-PLC\(\gamma\) signaling in primary human T cells in vitro. We go on to show the efficacy of glucocorticoid therapy in combination with a PLC\(\beta\) inhibitor in a mouse model of TSS in vivo. These findings have implications for our understanding of the mechanism of action of glucocorticoids. They may point to novel therapeutic strategies to counter glucocorticoid resistance.

Materials and Methods

Abs and reagents

The following Abs were purchased from Santa Cruz Biotecnology (Heidelberg, Germany): anti-Lck (3A5), anti–\(\beta\)-actin, anti-PLC\(\beta\)1 (D-8),...
anti-PLCβ2 (Q-15), anti-PLCβ3 (C-20), and anti-pTyr (PY20). Anti-phospho-Zap70 (Tyr319)/Syk (Tyr522), anti-phospho-Src (Tyr410), anti-phospho-LAT (Tyr171), and the PI3K inhibitor LY294002 were purchased from Cell Signaling Technology (Beverly, MA). Anti-PLCγ1 (2B1) was purchased from Abcam (Cambridge, MA). Anti-CD3 and anti-CD28 were purchased from Sanquin (Amsterdam, The Netherlands). PMA, ionomycin, staphylococcal enterotoxin B (SEB), and the PLCβ inhibitor U-73122 were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). PHA-M was purchased from Roche (Mannheim, Germany).

Mice

Eight-week-old female C57BL/6 wild-type mice were purchased from Harlan (Boxmeer, The Netherlands) and housed in pathogen-free conditions. All mice received a single i.p. injection with a total volume of 500 μl. Depending on the group, mice received 20 μg dexamethasone and/or 10 μg U-73122. All mice received 8.8 μl DMSO, 100 μg SEB. Twenty-four hours later, the mice were euthanized with carbon dioxide. Blood was drawn via intracardiac puncture, and the spleen was removed. Cytokine levels were determined using a Cytometric Bead Array (BD Bioscience, Breda, The Netherlands).

Cell culture

Human PBLs were isolated from whole blood of healthy volunteers by Ficoll-Isopaque density-gradient centrifugation. After washing, monocytes were separated from lymphocytes by Percoll density-gradient centrifugation. The lymphocytes were cultured in IMDM (Life Technologies, Verviers, Belgium) supplemented with 10% heat-inactivated FCS. For proliferation, experimental cells were stimulated for 24 h. In all experiments, lymphocytes were stimulated with PHA (10 μg/ml), SEB (100 ng/ml), or a combination of PMA (10 ng/ml) and ionomycin (250 ng/ml). Proliferation was measured by [3H]thymidine-incorporation assay.

RNA extraction and quantitative RT-PCR

Pelleted cells were dissolved in 500 μl TRIzol reagent. After adding 250 μl chloroform, tubes were spun, and the aqueous phase was transferred. RNA was precipitated by adding 250 μl isopropanol. RNA was washed in 70% EtOH, dried, and dissolved in H2O. For cDNA synthesis, 1 μg RNA was transcribed using RevertAid (Fermentas). Quantitative RT-PCR was performed using SYBR Green (QIAGEN), according to the manufacturer’s protocol, on a Bio-Rad iCycler using specific primers for the mRNA of interest.

Western blot

Samples for Western blot were made by treating 5 × 10^6 lymphocytes for 10 min with 10 μM dexamethasone, followed by 10 min of stimulation. Cells were pelleted and lysed in lysis buffer (0.1% Nonidet P-40, 50 mM Tris HCl, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 200 mM Na3VO4, 10 mM NaF) containing protease inhibitors (Roche) for 20 min on ice. Samples were spun down, and sample buffer was added to the supernatant and heated to 95°C for 5 min, cooled on ice, and sonicated. Samples were blotted overnight at 4°C in buffer containing 20% methanol on a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was blocked with 5% BSA for 1 h at room temperature (RT), followed by overnight incubation with the primary Ab at 4°C.

Immunoprecipitation

For immunoprecipitation experiments, 10 × 10^6 cells were used per condition. Cells were pelleted and lysed as described above. The supernatant was precleared by adding 10 μl Protein A/G UltraLink resin (Pierce Biotechnology, Rockford, IL) for 30 min at 4°C. Beads were spun down and discarded. Samples were incubated with 2 μg primary Ab and 25 μl beads for 2 h at 4°C. Beads were washed with PBS, and sample buffer was added and heated to 95°C for 5 min.

Immunohistochemistry

A total of 2 × 10^6 PBMCs was plated in a 12-well plate. Cells were pretreated for 30 min with 1 μM dexamethasone or with 0.5 μM U-73122 and then stimulated by adding SEB (100 ng/ml) for 1 h. Thirty minutes before the end of the experiment, cells were transferred to wells containing glass cover slips precoated with poly-l-lysine (0.01 [w/v] in H2O). The cells were fixed for 10 min in 4% paraformaldehyde at RT and stained by incubating with the primary antibody for one hour at room temperature, followed by incubation with the secondary Ab for 1 h at room temperature.

Small interfering RNA transfection

Two independent small interfering RNAs (siRNAs) targeting GNA11 (siRNA #1: CGACAGAUAUCUACUUU, siRNA #2: GCAUCAGAGUAUCCCUU), GNAq (siRNA #1: UCGUUCUAUCAUCAUCG, siRNA #2: AUACUUCUACAGAUCUCCG, and siPLCβ2 (siRNA #1: GCUCUACUAUCAUGUGAAG, siRNA #2: CCUGACCACAGAGAUU) were purchased from Ambion Applied Biosystems (Austin, TX). Lynphocytes were transfected by electroporation using Amaxa’s Nucleofector (Lanza, Cologne, Germany).

A total of 10 × 10^6 lymphocytes was electroporated per condition, according to protocol, using 30 pmol siRNA and the Human T Cell Nucleofector Kit for unstimulated T cells (Lanza, Cologne, Germany). The media were replaced after 4 h. Experiments were performed 48 h posttransfection.

Quantification and data analysis

Densitometric analysis of Western blot was performed using ImageJ (National Institutes of Health, Bethesda, MD). Data were analyzed using Excel (Microsoft), FlowJo (TreeStar, San Carlos, CA), and Prism (GraphPad Software, La Jolla, CA) software.

Results

T cell-activating signals that bypass Lck cause steroid resistance

Glucocorticoids inactivate canonical TCR signaling by dissociating the GR from a TCR-associated complex that contains Lck. This leads to inactivation of canonical TCR signaling, including PLCγ. PLCγ plays a critical role in the activation of Ca2+ signaling and activation of PKC. To test the hypothesis that inhibition of T cell activation by glucocorticoids depends on inactivation of Lck, we used different T cell stimuli to test their relative sensitivity to glucocorticoid-mediated inhibition of T cell proliferation. We used the plant lectin PHA to strongly activate canonical, Lck-dependent TCR signaling. PHA binds CD3 directly and nonspecifically (9), resulting in the potential activation of all T cells. Treatment of primary human peripheral blood leukocytes (PBLs) with 10 μg/ml PHA resulted in a strong activation of T cell proliferation that was sensitive to treatment with the synthetic glucocorticoid analog dexamethasone (Fig. 1B, figure shows relative proliferation. Mean ± SE of absolute proliferation of PHA-stimulated T cells without dexamethasone: 94,214 ± 7,281 cpm). Next, we completely bypassed Lck signaling by direct activation of Ca2+ signaling with 250 ng/ml ionomycin and PKC with 10 ng/ml PMA. Treatment with PMA/ionomycin resulted in complete resistance of proliferating T cells to dexamethasone, even at very high doses (Fig. 1B, mean ± SE of absolute proliferation of PMA/ionomycin-stimulated T cells without dexamethasone: 74,154 ± 1,190 cpm). These results suggest that the inhibitory effects of glucocorticoids on T cell proliferation completely depend on a nontranscriptional role for the GR and may be related to inactivation of Lck. To further address this question, we used the superantigen SEB. SEB not only activates canonical Lck-PLCγ signaling, it was also reported to activate a noncanonical G protein–PLCβ–dependent signaling pathway (8). If the effects of glucocorticoids depend on inhibition of Lck, this predicts that superantigen-induced T cell proliferation is only partially responsive to glucocorticoids. The hypothesis predicts that G protein–mediated activation of PLCβ is insensitive to the effects of glucocorticoids and, therefore, still allows for PLCγ-independent activation of Ca2+ signaling and activation of PKC (Fig. 1A). Treatment of PBLs with 100 ng/ml SEB resulted in an intermediate level of steroid resistance (Fig. 1B, mean ± SE of absolute proliferation of SEB-stimulated T cells without dexamethasone: 52,633 ± 1,664 cpm). This is consistent with the hypothesis that glucocorticoids inhibit SEB-induced activation of Lck-PLCγ signaling but not G protein–PLCβ signaling.
To examine whether glucocorticoids indeed inactivate both PHA- and SEB-mediated activation of Lck and canonical TCR signaling, we added 10 μM dexamethasone to PHA- and SEB-stimulated PBLs and determined phosphorylation of Lck and downstream signaling mediators ZAP70, LAT, and PLCγ. This experiment showed that dexamethasone inhibits both PHA- and SEB-mediated Lck signaling (Fig. 1C). This suggests that SEB-induced steroid resistance involves a bypass of the need for Lck-PLCγ signaling in T cell activation.

We reported previously that treatment with dexamethasone causes the disruption of a TCR-associated complex that contains both Lck and the GR (2, 3). To investigate whether a similar mechanism is responsible for the inhibition of SEB-stimulated signal transduction, PBLs were stimulated with SEB and treated
SEB causes steroid insensitivity through PLCb versus dexamethasone + U73122).

We used the PLCb inhibitor U73122 to investigate whether activation of PLCb by SEB is responsible for the relative insensitivity to dexamethasone (10). Treatment of SEB-stimulated PBLs with either U73122 (0.5 µM) or dexamethasone (10 µM) partially inhibited SEB-induced T cell proliferation. The combination of dexamethasone and U73122 showed an additive effect on T cell proliferation (Fig. 2A, p < 0.001, dexamethasone versus dexamethasone + U73122), suggesting that both inactivate independent signaling pathways. This experiment strongly suggests that SEB-induced activation of PLCb partially bypasses the inhibitory effects of glucocorticoids on Lck-PLCb signaling. Therefore, we used nuclear translocation of NFAT1 as a read-out of downstream TCR signaling. NFAT1 is translocated to the nucleus of activated T cells downstream of PLC-mediated Ca2+ signaling (11). If activation of PLCb bypasses glucocorticoid-mediated inhibition of Lck-PLCb signaling, this predicts that dexamethasone only partially prevents NFAT1 translocation in SEB-treated PBLs and that inhibition is complete if dexamethasone is combined with U73122. We found that PHA-mediated NFAT1 translocation was very sensitive to dexamethasone. In contrast, dexamethasone only partially prevented nuclear translocation of NFAT1 in SEB-treated PBLs, and translocation was completely prevented by dexamethasone/U73122 treatment (Fig. 2B, 2C, p < 0.001, dexamethasone versus dexamethasone + U73122).

**SEB-induced steroid resistance depends on PLCb signaling**

We used the PLCb inhibitor U73122 to determine whether activation of PLCb by SEB is responsible for the relative insensitivity to dexamethasone (10). Treatment of SEB-stimulated PBLs with either U73122 (0.5 µM) or dexamethasone (10 µM) partially inhibited SEB-induced T cell proliferation. The combination of dexamethasone and U73122 showed an additive effect on T cell proliferation (Fig. 2A, p < 0.001, dexamethasone versus dexamethasone + U73122), suggesting that both inactivate independent signaling pathways. This experiment strongly suggests that SEB-induced activation of PLCb partially bypasses the inhibitory effects of glucocorticoids on Lck-PLCb signaling. Therefore, we used nuclear translocation of NFAT1 as a read-out of downstream TCR signaling. NFAT1 is translocated to the nucleus of activated T cells downstream of PLC-mediated Ca2+ signaling (11). If activation of PLCb bypasses glucocorticoid-mediated inhibition of Lck-PLCb signaling, this predicts that dexamethasone only partially prevents NFAT1 translocation in SEB-treated PBLs and that inhibition is complete if dexamethasone is combined with U73122. We found that PHA-mediated NFAT1 translocation was very sensitive to dexamethasone. In contrast, dexamethasone only partially prevented nuclear translocation of NFAT1 in SEB-treated PBLs, and translocation was completely prevented by dexamethasone/U73122 treatment (Fig. 2B, 2C, p < 0.001, dexamethasone versus dexamethasone + U73122).

**SEB causes steroid insensitivity through PLCb2**

It was shown that SEB activates PLCb1 in the Jurkat T cell line. To determine which isoform of the PLCb family is responsible for the SEB-induced steroid insensitivity in primary human lymphocytes, we used Western blot to visualize the different PLCb isoforms that are expressed in human T cells (Fig. 3A). In contrast to Jurkat cells, primary human T lymphocytes express PLCb2, rather than PLCb1, and express low levels of PLCb3. To examine whether activation of PLCb2 is responsible for SEB-induced steroid re-

**FIGURE 2.** PLCb inhibitor U73122 prevents SEB-mediated glucocorticoid resistance. (A) Effect of dexamethasone, PLCb inhibitor U73122, and combination treatment on SEB-induced T cell proliferation. PBLs were stimulated with SEB (100 ng/ml) for 24 h in the presence or absence of inhibitors, as indicated. Proliferation was analyzed by [3H]thymidine-incorporation assay. (B) Effect of various treatments on nuclear translocation of NFAT1. PBLs were pretreated for 30 min with 1 µM dexamethasone or with 0.5 µM U-73122, followed by 1 h of SEB (100 ng/ml) stimulation. NFAT1 localization was visualized by immunohistochemistry. Each bar represents the average percentage of cells with nuclear NFAT1 staining as counted per three images at ×200 magnification. (C) Representative images of the NFAT1 staining quantified in (B). Original magnification ×200. NFAT1 staining is visible as a bright green staining, which accumulates in the nucleus in a proportion of the SEB-activated cells (arrows). Treatment with a combination of dexamethasone and U73122 maximally reduces nuclear translocation of NFAT1, see quantification in (B). Data in (A) and (B) are mean ± SE. *p < 0.05, **p < 0.001.

**FIGURE 3.** SEB-mediated glucocorticoid resistance is PLCb2 dependent. (A) Expression of PLCγ and different PLCb isoforms in human CD4 T cells, Jurkat cells, 3T3-L1 cells, and HEK293 cells indicates that PLCb2 is the predominant isoform expressed in primary human T cells. Western blot analysis of the presence of the different isoforms in samples with an equal amount of total protein from different cell types. (B) Western blot showing the efficiency of the two PLCb2 siRNAs in knocking down PLCb2 compared with cells transfected with control siRNA. (C) Effect of dexamethasone and PLCb2 siRNAs on SEB-mediated proliferation compared with the effect of dexamethasone on PHA-mediated proliferation. Cells were transfected with siRNA and allowed to recover for 2 d before stimulation and dexamethasone treatment. Proliferation was measured after 24 h of treatment by [3H]thymidine-incorporation assay. Data in (C) are mean ± SE. *p < 0.05, **p < 0.01.
sistance, we knocked down PLCβ2 in PBLs using two siRNAs (Fig. 3B). Indeed, knocking down PLCβ2 levels reduces SEB-induced proliferation and restores steroid sensitivity in primary human PBLs to levels similar to PBLs stimulated with PHA (Fig. 3C, figure shows relative proliferation; mean ± SE for absolute proliferation: PHA: 23,137 ± 397; SEB [control siRNA]: 16,570 ± 1,155). These results show that PLCβ2 is the predominant isoform expressed in primary human PBLs and support the hypothesis that SEB bypasses glucocorticoid-mediated inhibition of Lck-PLCγ signaling by activating PLCβ2.

**SEB-induced steroid resistance depends on activation of Gaq**

PLCβ is activated by members of the Gaq family of G proteins (12), such as Gaq11 (13) and Gaq (14). It was suggested that Gaq11 acts upstream of PLCβ activation in Jurkat T cells (8). We first used quantitative RT-PCR to examine the expression of both Gaq and Gaq11 in Jurkat cells and primary human CD4+ T lymphocytes using testis as a positive control for Gaq11 and monocytes as a positive control for Gaq (Fig. 4A). This showed that Gaq is the predominant Ga isoform expressed in both Jurkat cells and primary human T lymphocytes.

To further elucidate which G protein is involved in SEB-stimulated PLCβ2 activation, we transfected PBLs with siRNA targeting Gaq11 or Gaq. Knock-down efficiency of both Gaq and Gaq11 was determined by quantitative RT-PCR (Fig. 4B). Consistent with the low expression of Gaq11 in primary human T cells, siRNA against Gaq11 did not affect proliferation in SEB-stimulated PBLs (Fig. 4C, figure shows relative proliferation; mean ± SE for absolute proliferation; PHA only: 13,271 ± 782, SEB only [control siRNA]: 9,199 ± 459). In contrast, siRNA against Gaq reduced SEB-induced proliferation, showing that SEB-induced proliferation partially depends on Gaq signaling. This suggests that Gaq, and not Gaq11, acts upstream of PLCβ2 in primary human T cells. Gaq siRNA sensitized SEB-treated cells to treatment with dexamethasone. This is consistent with a role for Gaq signaling in mediating SEB-induced steroid resistance.

**SEB-treated mice are responsive to dexamethasone-PLCβ inhibitor combination treatment**

To test whether SEB-induced PLCβ activation is responsible for the relative inefficacy of glucocorticoids in the treatment of TSS, we injected mice with SEB and treated them with dexamethasone (20 μg/mouse, ~1 mg/kg), U73122 (10 μg/mouse, ~0.5 mg/kg), or both. Mice were injected i.p. with 100 μg SEB. In the same injection, mice received solvent, 20 μg dexamethasone (~1 mg/kg), 10 μg U-73122, or combination treatment. We measured the serum levels of TNF-α, IL-6, and IFN-γ at 8 and 24 h after treatment (Fig. 5A, 5B), as well as spleen weight (Fig. 5C) at 24 h, as a read-out of the response to treatment. Treatment with dexamethasone had little effect on serum cytokines. Dexamethasone did not significantly affect cytokine production at 8 h after treatment, and only IFN-γ was significantly reduced at 24 h. Treatment

![FIGURE 4](http://www.jimmunol.org/) SEB-mediated glucocorticoid resistance depends on Gaq11 signaling. (A) Quantitative RT-PCR for Gaq11 and Gaq in primary human CD4+ cells and Jurkat cells using testis and monocytes as respective positive controls. (B) Knockdown efficiency of Gaq11 and Gaq in PBLs, as measured by quantitative RT-PCR. (C) Effect of dexamethasone and Gaq11/Gaq siRNAs on SEB-mediated proliferation compared with the effect of dexamethasone on PHA-mediated proliferation. Cells were transfected with siRNA and allowed to recover for 2 d before stimulation and dexamethasone treatment. Proliferation was measured after 24 h of treatment by [3H]thymidine-incorporation assay. Data are mean ± SE. **p < 0.01, ***p < 0.001.
with U-73122 did not affect serum cytokine production at either time point. In contrast, dexamethasone/U-73122 (Fig. 6) combination treatment significantly reduced the production of all three cytokines at both time points. The same effect was seen for spleen weight. These data show that U-73122 sensitizes SEB-treated mice to treatment with dexamethasone.

FIGURE 5. The PLCβ inhibitor U73122 sensitizes SEB-injected mice to treatment with dexamethasone. (A) Serum cytokine levels 8 h after treatment with SEB. (B) Serum cytokine levels 24 h after treatment with SEB. (C) Spleen weights. Data are mean ± SE. *p < 0.05, **p < 0.01.

FIGURE 6. A model of SEB-induced steroid resistance in primary human T cells. Canonical Lck-PLCγ signaling is inhibited by dexamethasone at the level of Lck. This inhibitory effect of glucocorticoids can be completely bypassed by downstream activation of TCR signaling at the level of Ca2+ (ionomycin) and PKC (PMA) signaling. SEB causes partial steroid resistance by activating dexamethasone-sensitive Lck-PLCγ signaling in addition to dexamethasone-insensitive Goq-PLCβ2 signaling. Combination of dexamethasone with siRNA against Goq, PLCβ2, or U73122 effectively blocks SEB-induced T cell proliferation because it blocks both pathways.
Discussion

The GR has long been thought to exert its effects primarily through the transcriptional control of glucocorticoid target genes. It has become increasingly clear that the GR also has a nontranscriptional role in modulating cellular signal transduction. Recently, we showed that, in the absence of ligand, the GR is associated with the TCR complex. Glucocorticoids dissociate the receptor from the TCR complex, leading to rapid inactivation of Lck, the kinase that activates canonical PLCγ-dependent TCR signaling (2, 3). Glucocorticoids are potent inhibitors of T cell proliferation, but the extent to which this is dependent on dissociation of Lck from the TCR complex or transcriptional regulation had not been determined. In this study, we show that T cells can be made completely steroid resistant by directly activating them at a level downstream of PLCγ by stimulating them with PMA (activates PKC) and ionomycin (activates Ca2+-signaling). This shows that glucocorticoids act at a level upstream of activation of PKC and Ca2+-signaling (Fig. 6).

If glucocorticoids act at the level of Lck-PLCγ signaling, this predicts that signals that bypass this signaling route may render cells partially steroid resistant. This is why we examined the effect of glucocorticoids on SEB-mediated T cell activation. It was reported that SEB activates PLCβ-dependent signaling in a Jurkat T cell line, in addition to Lck-PLCγ signaling (8). This is why we determined whether this PLCβ-mediated bypass of Lck-PLCγ signaling is responsible for SEB-induced steroid insensitivity that was described in human PBMCs (7). We find that primary human PBLs primarily express PLCβ2. Inhibition of PLCβ signaling with U73122 or specific depletion of PLCβ2 both reduced SEB-mediated T cell proliferation. A combination of PLCβ inhibition plus dexamethasone completely inhibited T cell proliferation. Thus, the partial steroid insensitivity of SEB-treated T cells is dependent on PLCβ signaling. This shows that the effects of glucocorticoids on T cell proliferation are reliant on inhibition of PLCγ-dependent signaling. Thus, the effects of glucocorticoids on T cell proliferation are largely dependent on nontranscriptional effects of the GR.

PLCγ signaling is often activated by members of the guanine-binding protein family. It was shown that SEB-activated PLCβ signaling in Jurkat T cells is not sensitive to pertussis toxin and is mediated by Gα11. We confirmed that SEB-mediated T cell proliferation is not sensitive to pertussis toxin in human PBLs (data not shown). However, we find very low levels of Gα11 in primary human PBLs; they express high levels of the closely related family member Goαq instead. Indeed, depletion of Goαq, but not Gα11, reduced SEB-mediated proliferation and restored glucocorticoid sensitivity. This suggests that Goαq acts upstream of PLCβ2 in primary human PBLs.

The mechanism of action of SEB-mediated steroid insensitivity may not be relevant only as a model to study genomic versus nongenomic glucocorticoid signaling, it may also help to improve therapy for patients with TSS. These patients primarily receive supportive therapy because no specific treatment exists. Corticosteroids are not recommended for TSS (see http://www.uptodate.com), because clinical evidence for their efficacy is lacking, and a single small retrospective study showed that steroids may alleviate some symptoms of TSS, but they do not affect mortality (15). The limited efficacy of glucocorticoids in TSS is likely related to the fact that superantigens induce steroid resistance, and mechanisms to alleviate this may help to improve therapy. Indeed, we find that SEB-treated mice respond only marginally to treatment with either dexamethasone or the PLCβ inhibitor U73122.

This may be explained, in part, by the dosage of U73122 used in this experiment, which, compared with other studies, is relatively low (16, 17). However, dexamethasone/U73122 combination treatment significantly reduced cytokine production in SEB-treated mice. A caveat of the in vivo study is that we did not directly assess measures of T cell activation; it cannot be excluded that the observed effects of dexamethasone and U73122 are partially explained by effects on innate immune cells, such as monocytes or macrophages. However, our in vitro studies suggest that the effects may be directly on TCR signaling.

In conclusion, we find that SEB activates a Goq-PLCβ2-dependent signaling pathway that bypasses Lck-PLCγ signaling. Inhibition of this PLCβ-signaling pathway restores glucocorticoid sensitivity to SEB-activated T cells. This strongly indicates that glucocorticoid-mediated inhibition of T cell proliferation is dependent on inhibition of Lck-PLCγ signaling. Our data suggest that dual PLCγ/β inhibitors or glucocorticoid/PLCβ inhibitor combination therapy may be useful in the treatment of TSS.

Disclosures

The authors have no financial conflicts of interest.

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The authors have no financial conflicts of interest.

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If glucocorticoids act at the level of Lck-PLCγ signaling, this predicts that signals that bypass this signaling route may render cells partially steroid resistant. This is why we examined the effect of glucocorticoids on SEB-mediated T cell activation. It was reported that SEB activates PLCβ-dependent signaling in a Jurkat T cell line, in addition to Lck-PLCγ signaling (8). This is why we determined whether this PLCβ-mediated bypass of Lck-PLCγ signaling is responsible for SEB-induced steroid insensitivity that was described in human PBMCs (7). We find that primary human PBLs primarily express PLCβ2. Inhibition of PLCβ signaling with U73122 or specific depletion of PLCβ2 both reduced SEB-mediated T cell proliferation. A combination of PLCβ inhibition plus dexamethasone completely inhibited T cell proliferation. Thus, the partial steroid insensitivity of SEB-treated T cells is dependent on PLCβ signaling. This shows that the effects of glucocorticoids on T cell proliferation are reliant on inhibition of PLCγ-dependent signaling. Thus, the effects of glucocorticoids on T cell proliferation are largely dependent on nontranscriptional effects of the GR. PLCγ signaling is often activated by members of the guanine-binding protein family. It was shown that SEB-activated PLCβ signaling in Jurkat T cells is not sensitive to pertussis toxin and is mediated by Gα11. We confirmed that SEB-mediated T cell proliferation is not sensitive to pertussis toxin in human PBLs (data not shown). However, we find very low levels of Gα11 in primary human PBLs; they express high levels of the closely related family member Goαq instead. Indeed, depletion of Goαq, but not Gα11, reduced SEB-mediated proliferation and restored glucocorticoid sensitivity. This suggests that Goαq acts upstream of PLCβ2 in primary human PBLs.

The mechanism of action of SEB-mediated steroid insensitivity may not be relevant only as a model to study genomic versus nongenomic glucocorticoid signaling, it may also help to improve therapy for patients with TSS. These patients primarily receive supportive therapy because no specific treatment exists. Corticosteroids are not recommended for TSS (see http://www.uptodate.com), because clinical evidence for their efficacy is lacking, and a single small retrospective study showed that steroids may alleviate some symptoms of TSS, but they do not affect mortality (15). The limited efficacy of glucocorticoids in TSS is likely related to the fact that superantigens induce steroid resistance, and mechanisms to alleviate this may help to improve therapy. Indeed, we find that SEB-treated mice respond only marginally to treatment with either dexamethasone or the PLCβ inhibitor U73122.