Prostaglandin E₂ Modulation of p59fyn Tyrosine Kinase in T Lymphocytes During Sepsis

Mashkoor A. Choudhry, Shahab Uddin and Mohammed M. Sayeed

*J Immunol* 1998; 160:929-935; ;
http://www.jimmunol.org/content/160/2/929

References

This article cites 46 articles, 19 of which you can access for free at: http://www.jimmunol.org/content/160/2/929.full#ref-list-1

Why The JI? Submit online.

- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Fast Publication! 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Prostaglandin E<sub>2</sub> Modulation of p59<sup>fyn</sup> Tyrosine Kinase in T Lymphocytes During Sepsis<sup>1</sup>

Mashkoor A. Choudhry,* Shahab Uddin,† and Mohammed M. Sayeed<sup>2*</sup>

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) has been implicated in the suppression of T cell IL-2 production and proliferation during burn and sepsis. The present study evaluated the potential intracellular mechanism of suppressed T cell responses by assessing the activation of p59<sup>fyn</sup> kinase in T cells from septic rats as well as the T cells incubated with PGE<sub>2</sub>. p59<sup>fyn</sup> is known to regulate T cell functions. Sepsis was induced in rats by implanting fecal pellets containing Escherichia coli (150 CFU) and Bacteroides fragilis (10<sup>8</sup> CFU) into the abdominal cavity. For the assessment of PGE<sub>2</sub> role in sepsis, a group of septic rats were treated with indomethacin, which inhibits endogenous PGE<sub>2</sub> synthesis. As assessed by immunoblotting or in vitro kinase assay, a more than 40% inhibition of p59<sup>fyn</sup> phosphorylation and kinase activity was observed in septic rat T cells compared with the T cells from sterile or control rats. A similar inhibition in p59<sup>fyn</sup> phosphorylation and kinase activity was observed in PGE<sub>2</sub>-treated T cells compared with the T cells incubated in the absence of PGE<sub>2</sub>. The septic-related suppression in p59<sup>fyn</sup> phosphorylation and kinase activity in T cells was prevented in rats treated with indomethacin. We observed that the inhibition in p59<sup>fyn</sup> activation in septic or PGE<sub>2</sub>-treated T cells was due primarily to a suppression in p59<sup>fyn</sup> phosphorylation and not due to alterations in p59<sup>fyn</sup> protein expression. These findings suggest that PGE<sub>2</sub> released during sepsis could contribute to the sepsis-related suppression in T cell proliferation by attenuating p59<sup>fyn</sup> phosphorylation and its kinase activity. The Journal of Immunology, 1998, 160: 929–935.

<sup>1</sup>Abbreviations used in this paper: PLC-γ, phospholipase C-γ; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; IP<sub>3</sub>, inositol 1,4,5-triphosphate; DAG, 1,2-diacylglycerol; PTK, protein tyrosine kinase; [Ca<sup>2+</sup>]<sub>i</sub>, intracellular [Ca<sup>2+</sup>]; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; IP<sub>3</sub>, inositol 1,4,5-triphosphate; DAG, 1,2-diacylglycerol; PTK, protein tyrosine kinase; [Ca<sup>2+</sup>]<sub>i</sub>, intracellular [Ca<sup>2+</sup>].

Copyright © 1998 by The American Association of Immunologists

0022-1767/98/$02.00

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup>This work was supported by National Institute of Health Grants GM53235-02 and GM32288-09, and by a fellowship to M.A.C. from the Dr. Ralph and Marion C. Medical Research Trust Grant.

<sup>2</sup>Address correspondence and reprint requests to Dr. Mohammed M. Sayeed, Burn and Shock Trauma Institute, Loyola University Chicago Medical Center, Maywood, IL 60513; and 3Department of Medicine, University of Illinois at Chicago, Chicago, IL 60612

Received for publication April 16, 1997. Accepted for publication October 1, 1997.

the activation of T cells following their stimulation with Ag culminates into their proliferation and the release of IL-2. IL-2, a lymphokine released primarily by T cells, is essential for a proper functioning of the immune system (1, 2). The process of T cell activation is complex and involves several surface receptors (3). The activation of the TCR-CD3 complex serves as the primary signal. The receptor complex consists of at least seven distinct polypeptide chains, two of which form a heterodimer. The heterodimer recognizes the Ag. The other five polypeptide chains, collectively called CD3, are thought to be involved in the receptor assembly (4). While the CD3 polypeptides are a part of the TCR complex, they may not directly participate in the transmission of signals following Ag recognition (5). The activation of T cells could be achieved via stimulation of TCR with Ag, lectins, or with the Abs against CD3 (6). The earliest biochemical events following TCR occupancy are the increase in protein tyrosine kinase (PTK) phosphorylation (7, 8), and the activation of phospholipase C-γ (PLC-γ) (9). Activation of PLC-γ hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into inositol 1,4,5-triphosphate (IP<sub>3</sub>) and 1,2-diacylglycerol (DAG) (5, 6, 10, 11). Whereas IP<sub>3</sub> stimulates Ca<sup>2+</sup> release from the intracellular stores, DAG activates the protein kinase C activity (12–15). An increase in intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) sustained for several hours precedes T cell activation and its subsequent proliferation activity (13, 14, 16–19).

A suppression in the T cells’ proliferative response and their production of IL-2 has been observed following sepsis, burn, and trauma (20–22). Recent studies from our and other laboratories have implicated a role of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in the sepsis-associated suppression of T cell proliferation and IL-2 production (23–26). Additionally, we have shown that PGE<sub>2</sub> suppression of T cell proliferation and IL-2 production could result from a suppression in Ca<sup>2+</sup> signaling (25). The altered Ca<sup>2+</sup> signaling could result from either a direct effect of PGE<sub>2</sub> on [Ca<sup>2+</sup>]<sub>i</sub> homeostasis or via an effect on upstream signaling events such as the phosphorylation of PTKs. June et al. (27) have suggested that an inhibition in PTK phosphorylation could prevent TCR-mediated signal transduction including the [Ca<sup>2+</sup>]<sub>i</sub> elevation and production of IL-2. Among the tyrosine proteins that are phosphorylated after TCR simulation, p59<sup>fyn</sup>, P56<sup>lck</sup>, and ZAP70 have been recognized as playing important roles in T cell signal transduction pathways (3, 28). P56<sup>lck</sup> is associated with CD4 and CD8 molecules (29), and is believed to be important in the generation of signals through these molecules. ZAP70 is associated with the ζ-chain of TCR (30). p59<sup>fyn</sup> has been proposed to be instrumental in TCR-mediated signaling (31). Several studies have shown that the overexpression of fyn protein results in enhanced TCR-mediated Ca<sup>2+</sup> mobilization and T cell IL-2 production (3, 28, 32–34). Rigley et al. (35) suggested that p59<sup>fyn</sup> can regulate Ca<sup>2+</sup> influx via a mechanism that is independent of PIP<sub>2</sub> hydrolysis. That p59<sup>fyn</sup> is an integral component of the TCR-mediated signaling pathways is indicated by the co-immunoprecipitation of p59<sup>fyn</sup> with TCR (31), and the modulation in the signaling cascade with manipulations in the levels of p59<sup>fyn</sup> in transgenic mice (32, 36). The present study evaluated the effects of PGE<sub>2</sub> on the phosphorylation and activation of...
For the assessment of PGE_2 effects on the phosphorylation and activation of p59<sup>fyn</sup> in T cells during sepsis, septic rats were treated with indomethacin to block the endogenous PGE_2 synthesis (23–26), and to monitor its effect on activation of p59<sup>fyn</sup> and its kinase activity toward its target substrate. Further, we ascertained PGE_2-related changes in the p59<sup>fyn</sup> autophosphorylation and its mediation of tyrosine phosphorylation of other proteins in T cells incubated with and without PGE_2, in vitro.

**Materials and Methods**

**Bacterial strains and reagents**

*Escherichia coli* (American Type Culture Collection (ATCC), Rockville, MD; 25922) and *Bacteroides fragilis* (ATCC 25285) were obtained from Dr. Kenneth D. Thompson, University of Chicago, Chicago, IL. Con A, PGE<sub>2</sub>, enolase, and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO. mAbs to p59<sup>fyn</sup> (residues between 85–206 of human Fyn protein) were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. Anti-phosphotyrosine mAbs were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-receptor CD3 Abs were purchased from Pharmingen, San Diego, CA. [32P]-ATP was obtained from DuPont NEN, Boston, MA. Molecular weight markers were purchased from Sigma and other reagents for the SDS-PAGE were obtained from Bio-Rad, Richmond, CA. Immobil P membrane (polyvinylidene fluoride) for the transfer of proteins was obtained from Millipore, Bedford, MA. Reagents needed for the preparation of lysis buffer, wash buffer, and kinase buffer were obtained from Sigma. Nylon wool was obtained from Polysciences, Inc., War- nington, PA. Ficoll-Paque was purchased from Pharmacia, Uppsala, Sweden.

**Animal model of sepsis**

Male Sprague-Dawley rats (225 to 250 g) obtained from Harlan (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used. Sepsis was induced by implanting 1-cm<sup>3</sup> presterilized fecal pellets containing *Escherichia coli* (150 CFU) and *Bacteroides fragilis* (10<sup>5</sup> CFU) into the rat abdomen (22). Rats implanted with sterilized pellets without the bacteria are referred to as sterile. A febrile response (>38°C), decreased sensibilities to touch, diarrhea, eye hemorrhage, and piloerection were characteristic findings in septic rats on days 1 to 3 postimplantation. Whereas no mortality occurred in sterile rats, approximately 45% of all septic rats died between days 1 and 2 after implantation. Rats were killed 48 h after implantation.

**Treatment of animals with indomethacin**

Indomethacin (2 mg/kg) was administered i.p. initially 2 h prior to implantation, and 24 and 36 h after implantation (25). There was no significant difference in the mortality in the septic rats treated with and without indomethacin.

**T cell preparation**

Rats were killed to remove their spleens. Splenic T lymphocytes were isolated using the method previously described (25). Briefly, spleens were gently ground to prepare a single cell suspension. The red and dead cells from the suspension were removed by density gradient centrifugation with Ficoll-Paque. Splenocytes appearing at the interface of Ficoll and the medium were collected. To obtain a pure T cell preparation, splenocytes were incubated with nylon wool-packed columns. These columns were prequiled with HBSS supplemented with 10 mM HEPES, 5% FCS, and 50 gm of gentamicin per ml. The column containing cells was incubated at 37°C for 50 to 60 min. T cells were obtained by eluting the columns with 30 to 40 ml of HBSS at a flow rate of 1 drop per second. Flow cytomteric analysis was performed to assess the purity of the CD3-positive cells using anti-CD3 Abs. In most of the preparation, 90 to 95% of the cells were CD3 positive (data not shown).

**Stimulation of T cells and lystate preparation**

T cells obtained from control, sterile, and septic rats were stimulated with Con A (10 μg/ml) or anti-CD3 Abs (1 μg/ml) for 180 s at 37°C. The stimulation was stopped by cell solubilization in a phosphorylation lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 100 mM NaF, 1 mM MgCl<sub>2</sub>, 10 mM Na<sub>2</sub>PO<sub>4</sub>, 200 μM Na<sub>2</sub>VO<sub>4</sub>, 0.5% Triton X-100, and 10% glycerol) on ice for 45 to 50 min. Lysates were centrifuged at 10000 rpm for 5 min at 4°C.

**Immunoprecipitation**

Lysates were incubated with mAbs to p59<sup>fyn</sup> protein for 1 h, and then the mixture was incubated with protein G-Sepharose beads for another 2 h (37, 38). These incubations were conducted at 4°C. The precipitates were washed three times in phosphorylation lysis buffer without added glycerol.

**Immunoblot**

As described elsewhere (37, 38), immunoprecipitated protein after the third wash was analyzed by SDS-PAGE and transferred to Immobilon membranes using a semi-dry Trans-Blot system (Bio-Rad). The membranes were saturated with blocking buffer (10 mM Tris, 150 mM NaCl, 0.05% Tween-20 supplemented with 10% BSA) for 1 h at room temperature or for 16 to 20 h at 4°C followed by an incubation with anti-phosphotyrosine Ab (1:1000 dilution) at 4°C. The membranes were washed three times with wash buffer (10 mM Tris, 150 mM NaCl, 0.05% Tween-20). The membranes were incubated with a secondary Ab conjugated with horseradish peroxidase (1:3000 dilution) followed by washing. After the final wash, membranes were probed using enhanced chemiluminescence dye, and phosphoproteins were autoradiographed.

**In vitro kinase assay**

This was performed using the method described previously (37, 38). After the final wash, immune complexes were collected and washed twice times with in vitro kinase buffer (50 mM Tris-Cl, pH 7.4, 10 mM MnCl<sub>2</sub>, 0.1% Triton X-100). After these washes, kinase assays were performed by incubating immune complexes first with 5 μg/ml of acid-treated enolase, and then for 30 min with 10 μCi of [32P]-ATP. These incubation were conducted at room temperature (28°C). Samples were analyzed on SDS-PAGE (9%) and the proteins were transferred to the Immobilon membrane. Phosphoproteins were analyzed by autoradiography and the intensity of the bands was assessed using densitometry.

**Reprobing the membranes**

Membranes were reprobed after stripping the Abs. For stripping, membranes were incubated with stripping buffer (65 mM Tris-Cl, pH 6.8, 100 mM 2-ME, 2% SDS). Membranes were saturated and immunoblotting was performed essentially following the protocol mentioned in immunoblot section. For the analysis of fyn we have used anti-fyn mAbs instead of anti-phosphotyrosine Abs.

**Results**

**Analyses of p59<sup>fyn</sup> protein**

T cells obtained from control, sterile, and septic rats were lysed in the lysis buffer. The total protein content in each sample was assessed and analyzed for the presence of p59<sup>fyn</sup> using the immunoblot technique. A representative immunoblot is shown in Figure 1A. No significant change in the p59<sup>fyn</sup> protein levels was observed in T cells obtained from the control, sterile, and septic groups of rats. The protein contents of p59<sup>fyn</sup> in T cells from control and experimental animals were also analyzed after immunoprecipitation of p59<sup>fyn</sup> from the cell lysate with anti-fyn Abs, and were not found to be different between the control and experimental groups of animals (Fig. 1B). Densitometric analyses of the blots from immunoprecipitated fyn proteins are shown in Figure 1C. There seemed to be a decreasing trend in the fyn protein content in T cells from septic and sterile compared with control rats; however, it was not found to be statistically significant.

**Tyrosine phosphorylation of p59<sup>fyn</sup> protein**

T cells obtained from control and experimental animals were stimulated with Con A for 3 min at 37°C and lysed. Fyn protein was immunoprecipitated from the cell lysates using 1 μg of anti-fyn Abs and analyzed by SDS-PAGE. Proteins were transferred to the membrane and the phosphorylation of fyn protein was assessed by immunoblotting with anti-phosphotyrosine Abs. A representative immunoblot showing the phosphorylation of p59<sup>fyn</sup> protein is shown in Figure 2A. No detectable phosphorylation of p59<sup>fyn</sup> was observed in unstimulated T cells (data not shown). The stimulation of T cells with Con A resulted in the tyrosine phosphorylation of...
p59<sup>fyn</sup> protein. There was no change in the tyrosine phosphorylation of p59<sup>fyn</sup> in sterile rat T cells compared with control rat T cells. In contrast, a significant decrease in the phosphorylation of p59<sup>fyn</sup> PTK was observed in the T cells obtained from septic rats compared with the sterile and control rats’ T cells. To assure that the observed inhibition in p59<sup>fyn</sup> phosphorylation was primarily due to the septic injury and not due to different amounts of protein loaded in the lanes, we stripped the blots and reprobed for the content of p59<sup>fyn</sup> protein in each lane using anti-fyn Abs. The results are shown in Figure 2B. There was no change in the p59<sup>fyn</sup> protein contents among the lanes. These data suggested that the attenuation in phosphorylation of p59<sup>fyn</sup> protein was the result of septic injury. Finally, p59<sup>fyn</sup> phosphorylation data blots were analyzed using densitometric analyses, and the data from four or more similar experimental groups were pooled. The control response was normalized to 1. As shown in Figure 2C, while there was no significant change in the p59<sup>fyn</sup> phosphorylation in T cells obtained from sterile rats compared with the control rats, a 30 to 40% inhibition in the phosphorylation of p59<sup>fyn</sup> was observed in T cells obtained from septic rats compared with control and sterile rats.

**Assessment of p59<sup>fyn</sup> tyrosine kinase activity**

These experiments evaluated whether or not the above observed inhibition in p59<sup>fyn</sup> tyrosine phosphorylation could affect its ability to phosphorylate enolase, a known substrate for Src kinases. Phosphoproteins were analyzed using in vitro kinase assay and are shown in Figure 3A. There was no detectable p59<sup>fyn</sup> autophosphorylation or fyn-mediated phosphorylation of enolase in unstimulated T cells. Con A stimulation of T cells resulted in p59<sup>fyn</sup> autophosphorylation as well as enolase phosphorylation by fyn. Both the p59<sup>fyn</sup> autophosphorylation and enolase phosphorylation by fyn in control T cells were not significantly different from sterile rat T cells. In septic rat T cells, a significant inhibition was observed in both the autophosphorylation of fyn as well as its ability to phosphorylate the exogenous substrate compared with the T cells from control and sterile rats. The data from densitometric analyses pooled from several blots are presented in Figure 3, C and D. The fyn autophosphorylation was inhibited by 40 to 50% in septic rat T cells (Fig. 3C). Similarly, a more than 40% inhibition in enolase phosphorylation by fyn was found in septic rat T cells compared with control and sterile rat T cells (Fig. 3D). For equal loading of fyn in various lanes, blots were stripped and reprobed. No significant change in the amount of fyn was detected in various lanes (Fig. 3B).

**Effect of indomethacin on Fyn kinase activity**

In these studies, T cells were stimulated with anti-rat CD3 or Con A. The blots showing the fyn autophosphorylation and enolase

---

**FIGURE 1.** p59<sup>fyn</sup> protein levels in control (lane 1), sterile (lane 2), and septic (lane 3) rat T cells, in total cell lysate (A); immunoblot from immunoprecipitated p59<sup>fyn</sup> protein (B); and densitometric analyses of T cells from control (n = 5), sterile (n = 4), and septic (n = 5) rats (C). Values (means ± SE) obtained from densitometric analyses were normalized to the maximum value, which was considered to be equal to 1.

**FIGURE 2.** p59<sup>fyn</sup> phosphorylation in Con A-stimulated T cells obtained from control (lane 1), sterile (lane 2), and septic (lane 3) rats (A); immunoblot with anti-fyn Abs showing equal loading of protein in all lanes (B); and densitometric analyses of T cells from control (n = 5), sterile (n = 4), and septic (n = 6) rats (C). Values (means ± SE) obtained from densitometric analyses were normalized as described in the legend to Figure 1. *, p < 0.025, sepsis vs control or sterile (ANOVA).
phosphorylation by fyn in T cells after their stimulation with Con A or anti-CD3 Abs are given in Figure 4, A and C. There was no apparent change in the pattern of autophosphorylation of fyn as well as fyn-mediated phosphorylation of enolase in T cells after their stimulation with Con A or anti-CD3. Moreover, the kinase activity of p59<sup>fyn</sup> in T cells obtained from sterile rats treated with and without indomethacin was not significantly different. As shown above, a significant inhibition in both fyn autophosphorylation as well as the enolase phosphorylation by fyn was observed in septic rat T cells after their stimulation with Con A or anti-CD3. Indomethacin treatment of septic animals significantly prevented the inhibition in fyn autophosphorylation as well as the enolase phosphorylation. The data presented in Figure 4, E and F represent the groups of T cells stimulated with Con A. As shown in the figures, sepsis resulted in a 40 to 50% inhibition of fyn autophosphorylation (Fig. 4E) and fyn-mediated enolase phosphorylation (Fig. 4F) compared with sterile rats. The inhibition in fyn autophosphorylation and its kinase activity was evidently prevented when the rats were treated with indomethacin. Protein-loading control of the blots is shown in Figure 4, B and D. Because we did not find any difference in the stimulatory effects of Con A or anti-CD3 on T cells' p59<sup>fyn</sup> phosphorylation, we presumed the effects of PGE<sub>2</sub> blockade on p59<sup>fyn</sup> phosphorylation would be similar whether the cells were stimulated with Con A or anti-CD3.

**Effect of PGE<sub>2</sub> on control rat T cell Fyn autophosphorylation and its kinase activity**

T cells from control rats were incubated for 2 h with or without PGE<sub>2</sub> (1 μM) at 37°C before their stimulation with Con A or anti-CD3. The effects of PGE<sub>2</sub> on fyn autophosphorylation and its phosphorylation of enolase are shown in Figure 5, A and C. Stimulation of control rat T cells with Con A or anti-CD3 resulted in increased autophosphorylation of fyn as well as its kinase activity. As shown in Figure 5, A and C, autophosphorylation of p59<sup>fyn</sup> induced by Con A or anti-CD3 was significantly suppressed in T cells incubated with PGE<sub>2</sub> compared with T cells incubated in its absence. Similarly, phosphorylation of enolase by fyn in PGE<sub>2</sub>-treated T cells was significantly lower than that observed in untreated T cells. Densitometric analyses of six or more experiments performed with anti-CD3 are shown in Figure 5, E and F. A nearly 40 to 50% inhibition of fyn autophosphorylation was observed in T cells treated with PGE<sub>2</sub> compared with the untreated T cells (Fig. 5E). Similarly a 50 to 60% decrease in enolase phosphorylation was observed by fyn from PGE<sub>2</sub>-treated T cells compared with control T cells (Fig. 5F). Figure 5, B and D shows the equal protein-loading controls in blots shown in Figure 5, A or C, respectively.

**Discussion**

The demonstration by us (25) and others (26, 39, 40) that PGE<sub>2</sub> attenuates [Ca<sup>2+</sup>]<sub>i</sub> signaling in T cells supports the concept that PGE<sub>2</sub>-mediated suppression of T cell proliferation could result either from a disturbance in the [Ca<sup>2+</sup>]<sub>i</sub> response per se or in a signaling component upstream from it. Recently, our studies have corroborated the aforementioned concept by showing that PGE<sub>2</sub>-related alterations in Ca<sup>2+</sup> signaling and the proliferative response in T cells from septic animals could be overcome by the treatment of cells with ionomycin (41). Similar effects of Ca<sup>2+</sup> ionophore on PGE<sub>2</sub>-exposed T cells have previously been reported (39, 40). The present study has implied that p59<sup>fyn</sup> activation could indeed be the signaling component upstream from the [Ca<sup>2+</sup>]<sub>i</sub> responses, which is adversely affected in the presence of PGE<sub>2</sub>. Our study shows that...
PGE$_2$-related suppression in p59$^{fyn}$ activation occurs both in control rat T cells exposed to PGE$_2$ as well as T cells harvested from septic rats. The suppression of the p59$^{fyn}$ response was apparently not due to a decrease in the tyrosine phosphorylation of p59$^{fyn}$ as well as its ability to phosphorylate tyrosine residues of other proteins. As anticipated, the sepsis-mediated suppression in T cell fyn autophosphorylation and its kinase activity was preventable with the treatment of animals with indomethacin. The indomethacin treatment of sterile animals was without any effect on fyn tyrosine phosphorylation or its kinase activity. These results clearly support the idea that sepsis may primarily cause a suppression in T cell tyrosine phosphorylation of fyn and its ability to phosphorylate its substrates, which in turn attenuates T cells' [Ca$^{2+}$]$_i$ signaling and the proliferative response.

Although various studies have shown the suppression in T cell proliferation during burn, sepsis, and trauma, the mechanism of the proliferative suppression during these injury states has remained largely unknown (26). Several lines of evidence suggested that the burn-, sepsis-, or trauma-associated inflammatory response is accompanied by increased production of PGE$_2$ from phagocytic cells and monocytes (42, 43). On the basis of our present findings, an increase in the PGE$_2$ levels in the various inflammatory conditions would seem to play a role in the T cell suppression through an attenuation in p59$^{fyn}$ activation followed by disturbances in Ca$^{2+}$ signaling.

The tyrosine phosphorylation of fyn triggers its enzymatic activity and leads to the phosphorylation of the substrate proteins including PLC-$\gamma$1 (28, 34). PLC-$\gamma$1 phosphorylation in turn hydrolyzes PIP$_2$ into IP$_3$ and DAG (44). The IP$_3$-mediated release of Ca$^{2+}$ from intracellular stores and the subsequent Ca$^{2+}$ influx from the extracellular space are well-established prerequisites for T cell activation, proliferation, and lymphokine production (3, 11–13, 28). An inhibition in the upstream event such as fyn activation could potentially attenuate the [Ca$^{2+}$]$_i$ signal via altered PLC-$\gamma$ and hence lead to T cell proliferative dysfunction. There is also a possibility that fyn may not interfere with PIP$_2$ hydrolysis but may directly affect Ca$^{2+}$ signaling using a hitherto unknown pathway suggested by some studies (35, 45).

It remains unknown whether the fyn inhibition is caused by a direct effect of PGE$_2$ or by PGE$_2$ interference with the mitogen-induced signal transduction. PGE$_2$ interaction with its receptor results in an elevation of intracellular cAMP via an up-regulation of adenylate cyclase (40). Previous studies have shown that cholera toxin and forskolin, which up-regulate adenylate cyclase and subsequently increase cAMP levels, inhibit the TCR-mediated signal via an attenuation in most proximal events of the cascade such as CD3-$\zeta$ (46) and PLC-$\gamma$ phosphorylation (10). Whether or not fyn attenuation in septic rat T cells or T cells pretreated with PGE$_2$ is affected by these PGE$_2$-mediated second messengers is not known. However, as observed in cholera toxin-treated T cells, a

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** p59$^{fyn}$ autophosphorylation and kinase activity in T cells obtained from sterile and septic rats with and without indomethacin treatment. A shows fyn autophosphorylation and enolase phosphorylation by fyn in T cells stimulated with Con A, sterile (lane 1); sterile + indomethacin (lane 2); sepsis (lane 3); sepsis + indomethacin (lane 4); immunoblot with anti-fyn Abs showing equal loading of proteins in all lanes (B). C shows fyn autophosphorylation and enolase phosphorylation by fyn in unstimulated T cells (lane 1) and T cells stimulated with anti-CD3, sterile (lane 2); sterile + indomethacin (lane 3); sepsis (lane 4); sepsis + indomethacin (lane 5); protein loading control of the blot is shown in D; densitometric analyses of blots showing p59$^{fyn}$ auto-phosphorylation (E) and enolase phosphorylation by p59$^{fyn}$ (F) in Con A-stimulated T cells from untreated sterile (n = 8) and sepsis (n = 11) groups, and indomethacin-treated sterile (n = 5) and sepsis (n = 6) groups. *, p > 0.050, untreated sterile vs treated sepsis.

indomethacin (lane 2); sepsis (lane 3); sepsis + indomethacin (lane 4); immunoblot with anti-fyn Abs showing equal loading of proteins in all lanes (B). C shows fyn autophosphorylation and enolase phosphorylation by fyn in unstimulated T cells (lane 1) and T cells stimulated with anti-CD3, sterile (lane 2); sterile + indomethacin (lane 3); sepsis (lane 4); sepsis + indomethacin (lane 5); protein loading control of the blot is shown in D; densitometric analyses of blots showing p59$^{fyn}$ auto-phosphorylation (E) and enolase phosphorylation by p59$^{fyn}$ (F) in Con A-stimulated T cells from untreated sterile (n = 8) and sepsis (n = 11) groups, and indomethacin-treated sterile (n = 5) and sepsis (n = 6) groups. *, p > 0.050, untreated sterile vs treated sepsis.
In summary, sepsis induced a 40 to 50% inhibition of T cell fyn phosphorylation and its kinase activity. This was similar to the inhibition of fyn kinase activity in PGE$_2$-exposed T cells. Indomethacin treatment of rats prevented the septic-related suppression of fyn phosphorylation and kinase activity in T cells. These observations indicate that PGE$_2$ released during sepsis could contribute to suppressed T cell proliferation and IL-2 production by inhibiting p59$^{fyn}$ activation. Because p59$^{fyn}$ activation precedes [Ca$^{2+}$], signaling in T cells, and our earlier studies showed indomethacin prevented the sepsis-related attenuation in T cell [Ca$^{2+}$], responses, our present findings suggest that the septic injury initially induces a p59$^{fyn}$ down-regulation to lead to the disturbances in the [Ca$^{2+}$], responses, lymphokine production, and proliferation in T cells.

**Acknowledgments**

We are indebted to Dr. Sarfraz Ahmad for assistance in implanting bacterial pellets in rats and to Dr. Kenneth D. Thompson of the University of Chicago for providing the bacteria used in this investigation. We gratefully acknowledge the technical assistance of Dr. Z. Ahmed and L. Amato.

**References**


