Cutting Edge: TCR Stimulation by Antibody and Bacterial Superantigen Induces Stat3 Activation in Human T Cells

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Recent data show that TCR/CD3 stimulation induces activation of Stat5 in murine T cells. Here, we show that CD3 ligation by mAb and Staphylococcal enterotoxin (SE) induce a rapid, gradually accumulating, long-lasting tyrosine, and serine phosphorylation of Stat3 (but not Stat5) in allogene-specific human CD4+ T cell lines. In contrast, IL-2 induces a rapid and transient tyrosine and serine phosphorylation of Stat3. Compared with IL-2, CD3 ligation induces a delayed Stat3 binding to oligonucleotide probes from the ICAM-1 and IL-2R with IL-2, CD3 ligation induces a delayed Stat3 binding to oligonucleotide probes from the ICAM-1 and IL-2R. The cells. Moreover, we provide evidence that TCR/CD3 and IL-2 induce Stat3 activation via distinct signaling pathways. The Journal of Immunology, 1999, 163: 1742–1745.

Stat proteins play a key role in the regulation of T cell growth and differentiation. Stat protein knock-out mice are either embryonic lethal (Stat3) (6) or exhibit various defects in proliferation and signal transduction (4, 7).

In contrast to the well-established role of Stat proteins in cytokine receptor signaling, little is known about the function of Stat proteins in TCR signaling. In a recent study, Welte et al. (8) reported that TCR/CD3 ligation triggered tyrosine phosphorylation and activation of Stat5 in murine T cells. CD3-mediated Stat5 tyrosine phosphorylation was mediated via the Src-family tyrosine kinase Lck and involved a transient interaction between the TCR and Stat5 in murine T cells.

Here, we show that staphylococcal enterotoxin A (SEA) and anti-CD3 mAb induced a rapid and accumulating tyrosine phosphorylation and DNA-binding of Stat3 (but not Stat5) in allogene-specific, human CD4+ T cell lines. Thus, our data support the hypothesis that TCR signaling involves Src kinase-sensitive activation of Stat proteins and suggest that murine and human T lymphocytes differ in their usage of Stat proteins in TCR signaling.

**Materials and Methods**

**Cells lines**

Allogene-specific CD4+ human T cell lines (9) selected for this study produced no IL-2 after TCR ligation, as judged by ELISA and RNase protection assay.

**Abs and other reagents**

Anti-phosphotyrosine mAb (4G10) was purchased from Upstate Biotechnology (Lake Placid, NY); anti-Stat3 and anti-Stat5 Abs were from Transduction Laboratories (Lexington, KY); anti-CD3 Ab (F101.01) was a kind gift from Dr. C. Geisler (Institute of Medical Microbiology and Immunology, University of Copenhagen, Denmark); anti-phosphoserine-Stat3 (S727), anti-phosphotyrosine Stat3 (Y705), and anti-phosphotyrosine Stat5 (Y694) were from New England Biolabs (Beverley, MA). Biotinylated DNA oligonucleotides (ICAM-1, IL-2Rα GASd/EBSd) were from DNA Technology (Aarhus, Denmark); ICAM-1, IL-2Rα GASd/EBSd were from DNA Technology (Aarhus, Denmark); ICAM-1, IL-2Rα GASd/EBSd were from DNA Technology (Aarhus, Denmark); ICAM-1, IL-2Rα GASd/EBSd were from DNA Technology (Aarhus, Denmark); ICAM-1, IL-2Rα GASd/EBSd were from DNA Technology (Aarhus, Denmark).
IL-2Rαa, 5’TTTCTTCTGGAAGTACC; and IL-2Rαb, 5’GGTACTTTCTAGAAAGAAA.

Cell surface Ag analysis

Cell surface expression was analyzed by flow cytometry (FACScan; Beckton Dickinson, Mountain View, CA).

Preincubation and stimulation of cells

T cells (9) were incubated in medium containing anti-CD3 Ab (5 μg/ml), IL-2 (500 U/ml), SEA, and SEE (1 μg/ml), wortmannin (5 μM), rapamycin (25 ng/ml), or PP1 (10 μM) for the indicated periods of time. Cells were preincubated with inhibitors 1 h before incubation with cytokine, anti-CD3 Ab, or SE. Before stimulation, cells were starved for 4 h in medium containing 5% FCS.

Oligonucleotide affinity purification

Precipitation with biotinylated oligonucleotides was conducted as described for immunoprecipitations. Purified oligonucleotide-binding proteins were boiled in reducing SDS sample buffer and analyzed by SDS-PAGE and immunoblotting, as described earlier (9).

Results

Anti-CD3 Ab and SEA induced rapid tyrosine phosphorylation of Stat3 in Ag-specific, human CD4+ T cell lines (Fig. 1, A and D). The kinetics of SEA- and anti-CD3 mAb-induced Stat3 tyrosine phosphorylation were essentially identical. Phosphotyrosine Stat3 was detected within 1 min and was gradually accumulated for up to 60 min. In contrast, IL-2-induced tyrosine phosphorylation of Stat3 showed a distinctly different activation pattern and reached a maximum within 3–10 min, followed by a gradual decline within 60 min (Fig. 1G). The serine phosphorylation of Stat3 induced by anti-CD3 Ab and SEA (Fig. 1, B and E) followed a pattern that resembled the tyrosine phosphorylation pattern. Again, IL-2 induced a significantly different Stat3 serine phosphorylation pattern (Fig. 1H). Reprobing the nitrocellulose membranes with an anti-Stat3 Ab verified that equal amounts of Stat3 were present in the samples analyzed. Immunoprecipitation of phosphorylated proteins and immunoblotting with an anti-Stat3 Ab confirmed that SEA induced a gradual increase in tyrosine-phosphorylated Stat3 reaching a maximum after 2 h (Fig. 2A), whereas IL-2 induced a rapid, but transient increase in tyrosine-phosphorylated Stat3.

Recently, TCR stimulation was shown to induce tyrosine phosphorylation of Stat5 in murine T cells (8). To address whether CD3 ligation also induced Stat5 tyrosine phosphorylation, human T cells were treated with anti-CD3 mAb and IL-2. IL-2-induced activation of Stat3 and Stat5 after 10 min, whereas TCR ligation for 1 h activated only Stat3 (Fig. 2B). Furthermore, we were unable to detect tyrosine phosphorylated Stat5 after 10 min of CD3 ligation (data not shown).

We utilized biotinylated DNA oligonucleotides from different Stat3-binding promoter elements to investigate whether SEA induced binding of Stat3 to relevant DNA sequences. SEA induced binding of Stat3 to DNA oligonucleotides from the ICAM-1 and the IL-2Rα promoter within 1–4 h. In contrast, IL-2-induced Stat3 binding to these probes within 30–60 min (Fig. 3, A and B). Moreover, IL-2 also induced Stat5 binding to a biotinylated c-fos probe, whereas SEA induced only Stat3 activation (data not shown), supporting our conclusion that SEA and IL-2 induce activation of different Stat proteins.

Four hours after binding of Stat3 to the ICAM-1 and IL-2Rα probes, an increase in the surface expression of ICAM-1 and IL-2Rα could be detected by flow cytometry, which peaked within 16–24 h (Fig. 3, C and D). In contrast, SEA incubation had little effect on the surface expression of IL-2Rγ and CD38 (data not shown).

Recent evidence suggests that Lck, an Src family tyrosine kinase, plays a critical role in TCR-mediated activation of Stat5 in murine T cells (8). Here, we utilized an Src kinase inhibitor (PP1) to address whether Src kinases also play a role in CD3-mediated activation of Stat5. At concentrations above 5 μM, PP1 almost completely blocked SE-induced tyrosine phosphorylation of Stat3, but had no inhibitory effect on IL-2-induced Stat3 activation (Fig. 4). SEA-induced serine phosphorylation of Stat3 was also inhibited by PP1 (data not shown). SEA-induced binding of Stat3 to the ICAM-1 probe was also profoundly inhibited by PP1, whereas rapamycin had only a weak inhibitory effect (Fig. 4B). Wortmannin (wort), an inhibitor of phosphatidylinositol-3 (PI-3) kinase, had no effect on SEA- and IL-2-induced DNA binding of Stat3 (Fig. 4B). SEA-induced up-regulation of ICAM-1 and IL-2Rα expression was profoundly inhibited by PP1, whereas rapamycin had only a weak inhibitory effect (Fig. 4, C and D).
here, we provide the first evidence that anti-CD3 Ab and SEA induced a rapid, gradually increasing tyrosine phosphorylation of Stat3 in allogene-specific, human CD4\(^+\) T cell lines. IL-2 induced a rapid, but transient tyrosine phosphorylation of Stat3, suggesting that TCR and IL-2R activate Stat3 through distinct pathways. This conclusion was supported by our observations that SEA-induced Stat3 activation was blocked by PP1, whereas IL-2-induced Stat3 activation was unaffected. Reversely, IL-2-induced Stat3 activation was inhibited by an IL-2R\(\alpha\) blocking mAb, whereas CD3-induced Stat3 activation was not (data not shown).

In a recent study, Welte et al. (8) reported that anti-CD3 mAb induced tyrosine phosphorylation of Stat5 in murine T cells. However, we did not detect any Stat5 tyrosine phosphorylation following CD3 stimulation in human T cells. Yet, IL-2 induced tyrosine phosphorylation of Stat5 in these T cells. It is possible that TCR stimulation activates different Stat proteins in man and mouse. An alternative explanation might be that different T cell populations (e.g., naive vs memory T cells) induce different sets of Stat proteins after TCR ligation.

Src tyrosine kinases, such as Lck, play a crucial role in the early steps of TCR-mediated signal transduction. Lck mediates tyrosine phosphorylation of immune receptor tyrosine-based activation motifs in the TCR, which resemble and might function as Stat protein docking sites. Welte et al. (8) provided strong evidence that Lck plays a critical role in TCR-mediated Stat5 activation in murine T cells. Here, we show that PP1, an Src kinase inhibitor, blocked CD3-induced Stat3 tyrosine phosphorylation and DNA binding. It remains unknown whether Stat3 becomes tyrosine phosphorylated directly or indirectly upon TCR stimulation. IL-2R ligation triggers tyrosine phosphorylation of Stat proteins through an activation of Jak3 and Jak1 (3). We were unable to detect activated Jak kinases (Jak1, Jak2, Jak3, and Tyk2) following TCR stimulation in human T cells (data not shown). This observation was in agreement with other reports that TCR stimulation did not trigger activation of Jak kinase activity (10). Differences between the data presented here and previous studies on tyrosine phosphorylation of Stat3 following TCR stimulation (11) may lie partly in the cells and detection systems used. Here, we used Abs against tyrosine phosphorylated Stat3 and Stat5, which are highly sensitive, to detect tyrosine phosphorylation.

**FIGURE 2.** CD3 ligation induces tyrosine phosphorylation of Stat3, but not Stat5. **A,** Cells were incubated with medium, SEA, or IL-2 for the indicated periods of time, precipitated with anti-phosphotyrosine Ab (4G10), and immunoblotted with anti-Stat3 Ab. **B,** Cells were incubated with medium, IL-2, or anti-CD3 Ab as indicated and immunoblotted with anti-phosphotyrosine Stat3 or anti-phosphotyrosine Stat5. Blots were re-probed with anti-Stat3 and anti-Stat5.

**FIGURE 3.** SEA induces Stat3 binding to DNA oligonucleotides and modulated expression of ICAM-1 and IL-2R\(\alpha\). **A** and **B,** Cells were incubated with medium, IL-2, or SEA, as indicated, precipitated with biotinylated oligonucleotides from the ICAM-1 or the IL-2R\(\alpha\) promoter, and immunoblotted with anti-Stat3 Ab. **C** and **D,** Cells were incubated with SEA for the indicated periods of time and examined for cell surface expression of ICAM-1 and the IL-2R\(\alpha\) by flow cytometry. MFI, mean fluorescence intensity.
Recent evidence suggest that TCR stimulation triggers Stat3 serine phosphorylation via an activation of mitogen-activated protein kinases in T cells (12). Using Abs against serine phosphorylated Stat3, Cantrell and colleague (5) observed serine phosphorylation of Stat3 following TCR stimulation. Our data confirm and extend these findings. Since PP1 inhibited TCR-mediated serine phosphorylation, our results suggest Src family kinases to be involved in both serine and tyrosine phosphorylation of Stat3 in human T cells.

As IL-2 induces Stat3 activation in T cells (9), we investigated the possibility that TCR-induced Stat3 activation was due to an autocrine loop involving IL-2. First, TCR ligation triggers Stat3 activation in T cells that do not produce IL-2 (as judged by RNase protection assay and ELISA). Second, TCR triggers a rapid phosphorylation of Stat3, but not Stat5, whereas IL-2 triggers a rapid phosphorylation of Stat3 and Stat5 in parallel. Third, an IL-2R blocking mAb inhibits IL-2-induced Stat3 activation without affecting TCR-mediated Stat3 phosphorylation. However, our data do not exclude the possibility that the accumulated increase in phosphorylated Stat3 was due to an autocrine, cytokine-mediated activation loop.

In conclusion, we provide the first evidence that CD3 ligation triggers rapid Stat3 tyrosine and serine phosphorylation via a PP1-sensitive pathway in human CD4\(^{+}\) T cell lines.

References