Interleukin-4 (IL-4) plays a pivotal role in the induction and maintenance of allergy by promoting Th2 differentiation and B cell isotype switching to IgE. Studies on STAT6-deficient mice have demonstrated the essential role of STAT6 in mediating the biological functions of IL-4. IL-4 induces tyrosine phosphorylation of STAT6, which in turn leads to transcription of IL-4-specific genes. In addition, serine phosphorylation of STAT6 has recently been reported. Here we study the functional role of STAT6 serine phosphorylation and the kinases and phosphatases involved. We show that inhibition of protein phosphatase 2A (PP2A) induces serine phosphorylation of STAT6 and severely inhibits DNA binding of STAT6. In contrast, IL-4-induced tyrosine phosphorylation of Janus kinase-1 and STAT6 is not affected, suggesting that PP2A acts downstream of Janus kinases in IL-4 signaling. In conclusion, we provide the first evidence that PP2A plays a crucial role in the regulation of STAT6 function.

IL-4 is an important cytokine, which regulates the growth, differentiation, and survival of a variety of cell types. Thus, IL-4 plays a key role in the differentiation of native CD4+ T cells into Th2 T cells and in the regulation of apoptosis and growth of B cells. Furthermore, IL-4 induces B cell immunoglobulin isotype switching to IgG1 and IgE (reviewed in Refs. 1 and 2). Stimulation of the IL-4 receptor complex by IL-4 results in the activation of multiple signaling pathways, one of which involves signal transducers and activators of transcription (STAT6). IL-4 induces rapid tyrosine phosphorylation of STAT6 by IL-4 receptor-associated Janus kinases (JAK-1 and JAK-3), which in turn leads to STAT6 dimerization and rapid translocation to the nucleus, where STAT6 acts as an activator of IL-4-specific gene transcription (reviewed in Refs. 2 and 3). Studies of STAT6-deficient mice have shown that STAT6 plays a critical role in IL-4 signaling and the induction of allergy (4, 5). Recent reports suggest that IL-4, in addition to tyrosine phosphorylation, also induces serine phosphorylation of STAT6, but it is not known which role serine phosphorylation plays in the regulation of STAT6 signaling and which kinases and phosphatases are involved (6, 7).

At present four major classes of serine/threonine-specific protein phosphatases (PPases) are known. These include two that are Ca2+-independent (PP1 and PP2A) and two that are Ca2+-dependent (PP2B (calcineurin) and PP2C). PP1 and PP2A are expressed ubiquitously in eukaryotic cells and are reportedly involved in several signaling pathways (reviewed in Refs. 8 and 9). Here, we provide the first evidence that PP2A regulates IL-4-mediated STAT6 signaling.

**EXPERIMENTAL PROCEDURES**

Cell Lines and Plasmid Construct—Antigen-specific human CD4+ T cell lines were obtained from healthy donors and have been described elsewhere (10). The cutaneous tumor T cell line MF200 is has been described (11). Jurkat T cell line J76.25.20 and J-TAg have been described previously (12). The STAT6 reporter construct (ST6-pGL3) driven by the firefly luciferase gene was made by ligating pGL3 basic (Promega, Madison, WI) with an oligonucleotide (sense, 5'-CCGACTTCCCA-AAGACGTGCTCTCCCAAGAACTCTCTCCTTATTTACAAGGAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
for 30 min at 37 °C. The activity of PP1/PP2A in vivo was tested as described previously (15). Percentage inhibition of PP1/PP2A activity by calyculin A was calculated according to the formula: percentage inhibition = [(cpm - blank) - (cal A cpm - blank) x 100%/cpm - blank)] where "cal A cpm" indicates the cpm of the test sample with calyculin A, “blank” indicates the cpm obtained from the sample without cell lysate, and “cpm” indicates cpm of the sample without calyculin A. PP1/PP2A activity index 100 was set at “percentage inhibition” in the sample without calyculin A.

Protein Extraction and Western Blotting—Following incubation in medium with or without inhibitors and stimulation with rhIL-4 the cells were pelleted rapidly, and the reaction was stopped by lysing the cells in ice-cold lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 10% glycerol, with the following inhibitors: 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 4 μM iodoacetamide, 5 mM EDTA, 10 mM NaF, 10 μM aprotinin). SDS sample buffer was added to the lysates, which were subsequently subjected to 10% SDS-PAGE and transferred onto nitrocellulose membranes. Preparations of cytoplasmic/nuclear extracts were conducted as described previously (16). Blots were evaluated by using enhanced chemiluminescence, stripped, and reprobed according to the manufacturer's manual (Amerham Pharmacia Biotech).

Oligonucleotide Affinity Purification of STAT6—Cytoplasmic extracts from T lymphoma cells were prepared as described above. Lysates were precleared with streptavidin-coated agarose beads (KEM-TEC, Copenhagen, Denmark) and incubated with biotinylated double-stranded STAT6 binding sequence from the IgE promoter (IgE3(5), 5'-Bio-CGACCTCCAAAGGCTTTCCAAAGAAGCTC-3') (13). STAT-DNA complexes were precipitated using streptavidin-coated agarose beads. Purified DNA-binding proteins were boiled in SDS sample buffer and analyzed by SDS-PAGE and Western blotting.

In Vitro Dephosphorylation—Cells were incubated with or without calyculin A for 60 min prior to lysis in buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 10% glycerol, without inhibitors). The lysates were left on ice for 20 min, and thereafter the insoluble parts were removed (13,000 x g, 10 min). Then, the lysates were incubated with or without 0.5 unit/ml purified PP1 or PP2A at 37 °C for 120 min. The reactions were stopped by adding SDS sample buffer and boiling the samples for 5 min.

Thin-layer Electrophoresis of Phosphoamino Acids—Thin-layer electrophoresis was conducted as described previously (17). Briefly, cutaneous tumor T cells (4 x 10^6 cells/sample) radiolabeled with [32P]orthophosphate and [32P]-labeled STAT6 were immunoprecipitated before electrophoresis was boiled and separated by SDS-PAGE. Pre-stained standards (Novex, San Diego) were used as molecular weight markers. Resolved profiles and molecular weight markers were transferred electrophoretically from SDS polyacrylamide gel onto a polyvinylidene difluoride membrane, and subjected to autoradiography overnight without a screen. [32P]-Labeled proteins of interest were cut out of the polyvinylidene difluoride membrane and incubated in 6 M HCl for 2 min at 100 °C. The samples were then vortexed followed by hydrolysis at 110 °C for 60 min and dried with a flow of nitrogen. The samples were then run on 20 x 20-cm cellulose k-2F plates (60 mA, 55 min) in the presence of o-phosphoamino acid markers. The markers were visualized by spraying the plate with ninhydrin and o-pthalaldehyde in UV light. The labeled phosphoamino acids were detected by autoradiography.

Reporter Assay—In cotransfection experiments 3 x 10^6 Jurkat J-Taq cells were transfected with 1 μg of ST6-pGL3 reporter construct, 2 μg of wild-type STAT6 expression vector (TPU538), 1 μg of internal control pCMV-LacZ, and 4 μl of DMRIE-C (Invitrogen). Cells were rested for 24 h before being pretreated without or with calyculin A (20 or 40 mM) for 1 h prior to stimulation with IL-4 (25 ng/ml) for 18 h. Luciferase and β-galactosidase activities were assayed according to the instructions of the Promega luciferase assay system, and the Invitrogen β-galactosidase assay kit, respectively.

RESULTS
To measure the effect of calyculin A on phosphatase activity in intact cells, T cell lines were incubated with calyculin A for 60 min at 37 °C in a humidified atmosphere and washed extensively prior to analysis for PP1/PP2A activity. As shown in Fig. 1A, calyculin A induced a concentration-dependent inhibition of PP1/PP2A activity. In contrast, the PP2B inhibitor, cyclosporin A, had no effect on PP1/PP2A activity even at concentrations that blocked CD3 antibody-induced proliferation (Ref. 18; data not shown). To investigate whether PP1 and/or PP2A are involved in the regulation of STAT6, T cells were incubated with phosphatase inhibitors as described above, and total cell lysates were subsequently analyzed by Western blotting using an anti-STAT6 antibody. As shown in Fig. 1B (lane 5), incubation with calyculin A induced a change in the electrophoretic mobility of STAT6. Similar results were obtained with other PP2A inhibitors (okadaic acid, endothall thioanhydride, Fig. 1B, lanes 3–4) but not with inhibitors of PP1 (Tau, Fig. 1B, lane 2) and PP2B (Cya, Fig. 1B, lane 6). Similar effects of calyculin A on STAT6 electrophoretic mobility were observed in antigen-specific CD4+ T cell lines and T cell lymphoma/leukemia cell lines, indicating that the effect of PP2A inhibition is not limited to a specific cell line (data not shown). Phosphoamino acid analysis of immunoprecipitated STAT6 showed that calyculin A induces a strong increase of serine phosphorylation of STAT6 (Fig. 1C), which is compatible with the induced change in electrophoretic mobility of STAT6 (Fig. 1B). In contrast to the strong effect on serine phosphorylation, threonine phosphorylation was barely detectable following calyculin A treatment, indicating that calyculin A selectively modulates serine phosphorylation of STAT6.

To further investigate the involvement of PP2A in the regulation of STAT6 phosphorylation, we studied the effect of purified phosphatases in vitro. Total cell lysates from cutaneous T cells (Fig. 1D, lanes 1–8) or Jurkat T cells (Fig. 1D, lanes 9–12) pretreated with or without calyculin A were incubated with purified PP1 or PP2A enzyme prior to analysis by Western blotting with anti-STAT6 antibody. As shown in Fig. 1D, purified PP2A almost completely blocked the calyculin A-induced shift in electrophoretic mobility of STAT6 (lane 4 versus 2 and lane 10 versus 12). In parallel experiments purified PP2A enzyme had no effect on IL-4-induced tyrosine phosphorylation of STAT6 in either cytoplasmic or nuclear extracts (data not shown). In contrast, purified PP1 had no effect on the calyculin A-induced mobility shift of STAT6 in cutaneous T cells (Fig. 1D, lane 6 versus 2) and Jurkat T cells (data not shown). Taken together these data indicate that inhibition of PP2A triggers serine-phosphorylation of STAT6 in vivo, which can be dephosphorylated by PP2A in vitro.

In an attempt to identify the serine kinase responsible for the serine phosphorylation of STAT6, T cells were incubated with inhibitors of candidate serine kinases prior to treatment with calyculin A. Inhibitors of serine/threonine kinases such as MEK (PD90859), p38 MAPK (SB203580), JNK (PD169316), caserin II-kinase (5,6-dichloro-1-β-d-ribofuranosyl benzimidazole), phosphatidylinositol 3-kinase (wortmannin, LY294002), and the broad-spectrum kinase inhibitor H7 had no effect on calyculin A-induced phosphorylation of STAT6 (Fig. 2, lanes 3–8 and data not shown). However, preincubation with staurosporine, a broad-spectrum inhibitor of serine/threonine-kinases (19), was able to inhibit a calyculin A-induced mobility shift of STAT6 (Fig. 2, lane 10), suggesting that an as yet unidentified, staurosporine-sensitive serine kinase is involved in the regulation of serine phosphorylation of STAT6.

Because STAT6 mediates IL-4-induced gene activation and plays a critical role in cytokine-induced IgE responses, we addressed the question of whether PP2A regulates IL-4-mediated STAT6 activation. Accordingly, T cells were preincubated with calyculin A prior to stimulation with IL-4, and total cell lysates were subsequently analyzed by Western blotting using an anti-pT STAT6 antibody. As shown in Fig. 3A (upper panel), IL-4 induced a strong tyrosine phosphorylation of STAT6. Preincubation with calyculin A did not affect the amount of IL-4-induced tyrosine-phosphorylated STAT6 but triggered a signif-
sificant change in the electrophoretic mobility of tyrosine-phosphorylated STAT6 (Fig. 3A, upper panel, lane 4 versus 3).

Stripping and reprobing of the membrane with antibodies directed against total STAT6 and STAT4 showed that calyculin A induced a similar shift in electrophoretic mobility of STAT6 in IL-4-stimulated (and unstimulated) T cells (Fig. 3A, middle panel, lanes 4 and 2), and that the electrophoretic mobility of STAT4 was unaffected by calyculin A (Fig. 3A, lower panel). As shown in Fig. 3B, preincubation with calyculin A did not have any effect on IL-4-induced JAK-1 activation.

To address whether PP2A modulates IL-4-induced nuclear translocation of STAT6, cells were treated with phosphatase inhibitors and analyzed for STAT6 distribution in the cytosolic and nuclear fractions. As expected, the PP2A inhibitor calyculin A induced a significant shift in electrophoretic mobility of cytosolic STAT6, whereas inhibitors of PP1 and PP2B did not (Fig. 4A, upper panel). The amount of IL-4-induced tyrosine phosphorylation of STAT6 in the cytosol was unaffected by pretreatment with protein phosphatase inhibitors (Fig. 4A, upper panel). In contrast, phosphotyrosine STAT6 was almost completely absent in nuclear extracts from calyculin A-treated cells (Fig. 4A, lower panel, lane 5 versus 4). Stripping and reblotting with an antibody against total STAT6 showed that STAT6 was present in the nuclear extracts and that IL-4 induced a significant increase in the amount of total STAT6 (Fig. 4A, lower panel, lane 4 versus 1). Calyculin A did not inhibit the IL-4-mediated increase of total STAT6 in the nuclear fraction (Fig. 4A, lower panel, lane 5 versus 2) but induced a shift in the electrophoretic mobility of STAT6 in nuclear extracts that was comparable (but not identical) to that seen in the cytosolic fraction (Fig. 4A, lane 5, lower versus upper panel). In contrast to the inhibition of phosphotyrosine STAT6 by calyculin A,
Inhibitors of PP1 and PP2B had no effect on phosphotyrosine STAT6 (and total STAT6) in nuclear extracts (Fig. 4A, lower panel).

To address whether calcineurin-induced serine phosphorylation influences the ability of STAT6 to bind promoter regions of IL-4 target genes, we took advantage of the oligonucleotide "fishing" assay (20). In this assay, biotinylated oligonucleotide probes representing the STAT6-binding site from the IgE promoter were used to precipitate promoter-binding proteins (13). As shown elsewhere (21) and confirmed in Fig. 4B (lane 3), IL-4 induced a strong binding of tyrosine-phosphorylated STAT6 from the cytoplasm to the IgE oligonucleotide sequence. Although calcineurin did not inhibit tyrosine phosphorylation of STAT6 in the cytosol (Fig. 4A), preincubation with calcineurin almost completely blocked the binding of tyrosine phosphorylated STAT6 to the oligonucleotide probe (Fig. 4B, lanes 2 and 4), suggesting that calcineurin-induced serine phosphorylation blocks the DNA binding function of STAT6. To investigate whether pretreatment with calcineurin also inhibits the transcriptional activity of IL-4-induced STAT6, we made a STAT6-responsive luciferase expression construct. As shown in Fig. 4C, luciferase expression induced after 18 h of IL-4 stimulation was completely inhibited by preincubation with calcineurin A (20 and 40 nM).

DISCUSSION

In the present study we provide the first evidence that protein phosphatases are involved in the regulation of STAT6. Inhibition of PP1/PP2A phosphatase activity by calcineurin A triggered serine phosphorylation and a shift in the electrophoretic mobility of STAT6. Two structurally different PP2A inhibitors, okadaic acid and endothall thioanhydride, also induced a significant shift in the electrophoretic mobility of STAT6, whereas inhibitors of PP1 (tautomycin) and PP2B (cyclosporin A) did not, suggesting that PP2A rather than PP1 (and PP2B) plays a key role in the regulation of STAT6. This conclusion is in accordance with our observation that treatment of cell lysates with purified PP2A restored the electrophoretic mobility of STAT6, whereas incubation with PP1 enzyme did not. Phosphoamino acid analysis of STAT6 showed that inhibition of PP2A leads to a strong increase in serine phosphorylation in vivo. In contrast, calycin A did not induce threonine phosphorylation, suggesting that
PP2A selectively regulates serine phosphorylation of STAT6.

In an attempt to identify the kinase responsible for serine phosphorylation of STAT6, we took advantage of cell-permeable inhibitors of candidate serine kinases. It has recently been shown that calyculin A induces MAPK (ERK1/2) activation through a PD98059-sensitive pathway in T cells (17, 22), and MAP and JNK kinases have been implicated in cytokine-induced serine phosphorylation of STAT1, STAT3, and/or STAT5 (23–25). Therefore, we explored the possible role of MAP and JNK kinase pathways in calyculin A-induced STAT6 phosphorylation. Despite an almost complete inhibition of ERK1/2 activation (data not shown), the MEK inhibitor PD98059 did not inhibit the effect of calyculin A on STAT6, indicating that the serine phosphorylation of STAT6 was not mediated via the MEK/MAPK pathway. In support of this conclusion, STAT6 lacks the PSXP MAPK consensus phosphorylation site found in STAT3 and STAT1 (7), and the PSP motif found in STAT5a and -b (23). Inhibitors of the P38 and JNK pathways also failed to inhibit the calyculin A-induced mobility shift of STAT6. In contrast, staurosporine inhibited the calyculin A effect on STAT6, suggesting that an as yet unidentified, staurosporine-sensitive kinase was responsible for calyculin A-induced serine phosphorylation of STAT6. This is in agreement with our previous findings that staurosporine blocks serine phosphorylation of STAT3 in response to PP2A inhibitors (17). We therefore hypothesize that the level of serine phosphorylation of STAT6 results from a balance between phosphorylation by a constitutively active, staurosporine-sensitive kinase and dephosphorylation by PP2A. This hypothesis is supported by our finding that STAT6 is a substrate for PP2A in vitro. Alternatively, PP2A may function as a negative regulator of a staurosporine-sensitive kinase.

Our observation that calyculin A did not inhibit IL-4-induced activation of JAK-1 and STAT6 indicates that PP2A is not involved in the initial signaling events following IL-4R ligation. This conclusion was in accordance with our finding that inhibition of PP2A activity did not inhibit IL-4-mediated translocation of STAT6 to the nucleus. Because we were unable to detect tyrosine-phosphorylated STAT6 in the nucleus of IL-4-stimulated cells, it appears that calyculin A treatment induced nuclear tyrosine dephosphorylation of STAT6. Alternatively, calyculin A might induce re-export from the nucleus of tyrosine-phosphorylated STAT6. A more trivial explanation could be that calyculin A directly or indirectly, interfered with antibody recognition of tyrosine-phosphorylated STAT6 isolated from the nucleus. However, our observation that antibody recognition of tyrosine-phosphorylated STAT6 obtained from the cytosolic fraction was unaffected by calyculin A strongly argues against this possibility.

It has been a matter of some controversy as to how serine phosphorylation modulates the function of STAT proteins. Thus, serine phosphorylation has been reported to increase or decrease the level of tyrosine phosphorylation and/or the transcriptional activity of STAT1, STAT3, and/or STAT5 (22, 26–30). Two recent papers reported that IL-4 induces serine phosphorylation of STAT6 (6, 7), but the authors were not able to ascribe any functional effect of serine phosphorylation on the STAT6 signaling pathway. Here we show that inhibition of PP2A induces serine phosphorylation and inhibition of both the DNA binding capacity and the transcriptional activity of STAT6.

Because a major site for serine phosphorylation lies within the known transactivation domain of STAT6 (7), it is possible that inhibition of STAT6 binding to the IgE promoter is caused by calyculin A-mediated serine phosphorylation of the transactivation domain. Studies are in progress to address this hypothesis. In conclusion, we have provided the first evidence that PP2A regulates cytokine-mediated STAT6 signaling.

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