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An L*XX*LL Motif in the Transactivation Domain of STAT6 Mediates Recruitment of NCoA-1/SRC-1*

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Signal transducer and activator of transcription 6 (STAT6) regulates transcriptional activation in response to interleukin-4 (IL-4)-induced tyrosine phosphorylation by direct interaction with coactivators. The CREB-binding protein and the nuclear coactivator 1 (NCoA-1), a member of the p160/steroid receptor coactivator family, bind independently to specific regions of STAT6 and act as coactivators. In this study we show that an L*XX*LL motif in the STAT6 transactivation domain mediates the interaction with NCoA-1. Peptides representing this motif as well as antibodies generated against this motif inhibited STAT6/NCoA-1 interaction in glutathione *S*-transferase pulldown assays. Peptides derived from the STAT6 transactivation domain adjacent to the L*XX*LL motif as well as antibodies against these peptides showed no inhibitory effect. Mutagenesis of the L*XX*LL motif eliminated the STAT6/NCoA-1 interaction *in vitro* and *in vivo*, supporting the specific role of this motif in NCoA-1 binding. Importantly, mutagenesis of the STAT-L*XX*LL motif strongly diminished the IL-4-regulated activation of the endogenous STAT6 target gene eotaxin-3. Taken together, these results indicate that the STAT6-L*XX*LL-binding motif mediates the interaction with NCoA-1 in transcriptional activation and represents a new potential drug target for the inhibition of the STAT6 transactivation function in allergic diseases.

STAT6[1] belongs to the STAT family of transcription factors that transmit signals from activated cytokine receptors to the nucleus. Following their obligatory tyrosine phosphorylation exerted by JAK kinases, STATs dimerize and move to the nucleus where they modulate transcription through binding to specific DNA sequence elements (1–3). STAT proteins share the same structure and functional domains, although they have distinct regulatory functions in the organism (4). STAT6 becomes activated in response to IL-4 and IL-13 and mediates most of the gene expression regulated by these cytokines. IL-4 regulates immune and anti-inflammatory responses. It promotes the differentiation of T helper (Th) precursors toward the Th2 lineage while inhibiting Th1 development. Furthermore, IL-4 triggers immunoglobulin class switching to the IgE isotype in B-cells. STAT6-deficient mice have defects in IL-4-mediated Th2 development and immunoglobulin class switching to IgE, demonstrating the essential role of STAT6 in these functions (5, 6). Recent studies (7, 8) have shown that the chemokine eotaxin-3 can be up-regulated by IL-4 and IL-13 in endothelial cells and dermal fibroblasts in a STAT6-dependent manner. Eotaxin-3 is a member of the family of CC chemokines, which are known to be potent chemoattractants for eosinophils. A STAT6-response element was identified in the promoter of the eotaxin-3 gene, characterizing this chemokine as a novel STAT6 target gene (8).

Control of gene expression is a dynamic and complex process. Gene transcription involves assembly of multiple transcription factors at the distal enhancer region and the basal transcriptional machinery, including RNA polymerase II, at the core promoter of target genes (9). According to the current model, gene expression involves the sequential assembly of an array of coregulatory proteins, including coactivators that are part of the chromatin-modifying complexes, possessing histone acetyltransferase activities (10). Coactivator complexes are recruited via specific protein/protein interactions, often mediated by specific interaction motifs in the transactivation domains of DNA-bound transcription factors or other coactivators.

The transactivation domain (TAD) of STAT6 was characterized as a modular, proline-rich region in the carboxyl terminus of the protein (11, 12). Two distinct transactivation functions, which cooperate in transcriptional activation, have been mapped to this domain (13). In earlier studies we reported that STAT6 recruits the coactivators p300/CBP and NCoA-1 via direct interaction with specific parts of its transactivation domain (14, 15). Both coactivators are required for transcriptional activation by IL-4 (14, 15). p300 and the CREB-binding protein CBP are large, highly related, multifunctional coactivators that share many structural and functional attributes and are referred to collectively as p300/CBP (16). p300/CBP function as coactivators for many DNA-binding transcriptional activator proteins, including nuclear receptors and other signal-regulated activators (17). p300/CBP have been shown to associate with other coactivators such as p/CAF (18) and members of the NCoA coactivator family also called the p160/steroid receptor coactivator (SRC) family (19, 20), thereby providing a platform for a variety of proteins participating in gene expression (16). The NCoA coactivators were identified as nuclear receptor-binding proteins, which enhance transcriptional activation by ligand-induced transcription factors (21). Three homologous factors, termed NCoA-1, also called SRC-1 (19, 22); NCoA-2, also called TIF2 or GRIP1 (23, 24); and NCoA-3, also called p/CIP, ACTR, or AIB1 (25–27), have been identified by

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[1] The abbreviations used are: STAT6, signal transducer and activator of transcription 6; GST, glutathione *S*-transferase; IL, interleukin; CBP, CREB-binding protein; NCoA, nuclear coactivator; SRC, steroid receptor coactivator; TAD, transcriptional activation domain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; RAR, retinoic acid receptor; DBD, DNA binding domain; GFP, green fluorescent protein; RT, reverse transcriptase; Th, T helper.

several laboratories. Subsequently a number of transcription factors have been shown to recruit these coactivators. It has been proposed that distinct classes of transcription factors selectively use specific coactivators and histone acetyltransferase activities for their function (28, 29). In accordance with this model, we have observed a specific role of NCoA-1, but not NCoA-2 or NCoA-3, for STAT6-mediated transactivation (15). Only NCoA-1 acts as a coactivator of STAT6. STAT6 directly contacts NCoA-1 via a small carboxyl-terminal part of its transactivation domain. A region between amino acids 213–462 in NCoA-1, which comprises the B part of the Per-Arnt-Sim domain and part of a serine/threonine region, mediates the interaction with STAT6. Overexpression of the STAT6-interacting domain of NCoA-1 inhibits transactivation by STAT6 in a transdominant manner, demonstrating the importance of NCoA-1 for STAT6 transactivation. In the present study we characterize the 56-amino acid spanning interaction domain in the STAT6-TAD that mediates the recruitment of NCoA-1. We have identified an L*XX*LL motif (where L is leucine and *X* is any amino acid) with a predicted α-helical structure in the shortest NCoA-1-interacting fragment of the STAT6-TAD. The α-helical L*XX*LL motif was originally found in a variety of coactivators, *e.g.* NCoA/p160/SRC members, p300/CBP, RIP-140. It is described as a signature motif, which mediates the recruitment of these proteins by the nuclear hormone receptors (30, 31). Specific L*XX*LL motifs of NCoAs mediate the ligand-dependent interaction with nuclear receptors, as well as interaction with p300/CBP. In this study we demonstrate that STAT6 recruits NCoA-1 via an L*XX*LL motif in its transactivation domain. We show that a peptide containing the L*XX*LL motif of STAT6, as well as antibodies raised against this peptide, are potent inhibitors of the interaction with NCoA-1 *in vitro*. Furthermore, mutagenesis of the STAT6-L*XX*LL motif abolishes the binding of NCoA-1 *in vitro* and *in vivo*. A STAT6 variant, in which the L*XX*LL motif is mutated, is less active in induction of a STAT6-dependent reporter gene. Moreover, the L*XX*LL mutant of STAT6 shows impaired induction of eotaxin-3 expression, indicating that recruitment of NCoA-1 by the STAT6-L*XX*LL motif is essential for full transactivation of this endogenous target gene. The L*XX*LL motif of STAT6 shows no sequence homology to the known coactivator motifs. A peptide containing the STAT6-L*XX*LL motif does not interfere with nuclear receptor/coactivator interactions, suggesting that this motif might represent a specific target for STAT6 inhibition. Our data indicate that NCoA-1 does not only use its own L*XX*LL motifs to contact DNA-bound factors and coactivators, but can also be recruited itself by an L*XX*LL motif of DNA-bound transcription factors, such as STAT6.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfections, and Luciferase Assays—293T cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 2 mM L-glutamine, penicillin/streptomycin. Transfections were performed by the calcium phosphate precipitation method. The following day, cells were induced with human IL-4 (10 ng/ml, PeproTech) or left untreated as indicated and lysed after a further 16 h of incubation. The human lung epithelial cell line NCI-H292 was grown in RPMI medium containing 10% fetal calf serum, 2 mM L-glutamine, penicillin/streptomycin. Cells were transfected with FuGENE 6 transfection reagent (Roche Molecular Biochemicals) according to the manufacturer's instructions. Cells were induced with human IL-4 (10 ng/ml) or left untreated and were harvested 40 h after transfection for luciferase assays. Luciferase and β-galactosidase activities were assayed as recommended by the manufacturer (Promega). Luciferase activities were normalized to the *lacZ* expression. At least three independent experiments were performed.

Plasmids—The reporter genes (GAL4-RE)3TK LUC, N4(STAT-RE)3 LUC, the *lacZ* expression plasmid (pCH110), the expression vectors for h-STAT6 (pXM-STAT6), and GAL4-DNA binding domain have been described previously (12). The expression vector for mutant STAT6 with the point mutations L802A,L805A (pXM-STAT6-mut) was generated from pXM-STAT6 by site-directed mutagenesis according to the protocol of Stratagene. The expression vectors encoding GFP-tagged wild type or mutant STAT6 variants were obtained by insertion of the corresponding STAT6 sequence generated by PCR into the *Xho*I and *Kpn*I sites of ECFP-C1 vector (CLONTECH). Expression vector for murine NCoA-1 was kindly provided by Joe Torchia (University of Western Ontario, London, Canada). The expression vectors for the GAL4-DNA binding domain fusion proteins containing residues 792–847 of wild type and mutant (L802A,L805A) STAT6 were generated by insertion of PCR fragments encoding the corresponding sequences into the *Eco*RI/*Nhe*I sites of pCMV-GAL4-DBD. pGEX-5X-1 was purchased from Amersham Biosciences. The expression vectors for wild type and mutant STAT6 (L802A,L805A) residues 642–847 fused to GST were generated by insertion of the corresponding region (generated by PCR) in-frame into the *Bam*HI/*Xho*I sites of pGEX-5X-1. The expression vector for GST-RAR has been described previously (32). To generate a bacterial expression vector for residues 213–462 of NCoA-1 fused to an amino- and carboxyl-terminal histidine tag, the PCR fragment encoding the corresponding region was inserted into the *Bam*HI/*Xho*I sites of pET30C (Novagen). Baculovirus expression vector for full-length GST- and histidine-tagged STAT6 was generated by introducing the GST sequence in the *Nde*I site of pFAST-BacHTb-STAT6 expression vector which contains full-length STAT6 sequences downstream of His_6. pFAST-BacHTb expression vector was purchased from Invitrogen. All constructs were verified by digestion. Inserts generated by PCR were additionally verified by sequence analysis.

Generation of STAT6 Peptides and Antibodies—Peptides comprising sequences 794–814 (peptide 1), 817–830 (peptide 2), and 830–847 (peptide 3) of STAT6 were obtained from Eurogentec. Antisera against the peptides were generated by Eurogentec according to standard procedure. Crude end bleedings after three booster injections were used as antisera.

GST Pulldown Assays—Recombinant cDNAs of NCoA-1 in the pCMXPL2 or NCoA-1 amino acids 213–462 in pET30C expression vector were transcribed and translated *in vitro* in reticulocyte lysates (Promega) in the presence of [^{35}S]methionine according to the manufacturer's instructions. GST and other GST-STAT6-TAD fusion proteins were expressed in *Escherichia coli*. GST-histidine-tagged full-length STAT6 fusion protein was expressed in Sf9 insects cells, infected with recombinant baculovirus. All GST proteins were purified with glutathione-Sepharose beads (Amersham Biosciences). For binding assays, GST fusions or GST alone (1.5–5 μg) bound to glutathione-Sepharose beads were incubated with radiolabeled or histidine-tagged proteins in 200 μl of binding buffer as described previously (33). Ligand-dependent interaction of GST-retinoic acid receptor α and NCoA-1 was investigated in the presence of all-*trans*-retinoic acid (10^{-6} M). For competition assay, binding was performed in the presence of antisera or peptides as indicated. After extensive washing, bound proteins were eluted and separated on SDS-PAGE. Radiolabeled proteins were visualized by fluorography. Histidine-tagged proteins were detected by Western blot using an antibody recognizing the histidine tag. Amounts and integrity of bound proteins were estimated after SDS-PAGE by Coomassie staining.

Coimmunoprecipitation Assays—293T cells were transfected with 3 μg of expression vectors encoding GFP-tagged wild type or mutant STAT6 variants, and 7 μg of expression vector for NCoA-1 per 10 cm-dish. 2 days after transfection the cells were lysed in NETN buffer as described previously (15). Cleared lysates were incubated with 2 μl of anti-GFP antisera (CLONTECH) for 2 h and for another hour with protein A/G-agarose beads. The immunoprecipitated proteins were separated by SDS-PAGE and analyzed by Western blotting with STAT6 antibody (BD PharMingen and Transduction Laboratories) and SRC-1 antibody (clone 1135, Upstate Biotechnology, Inc.).

Preparation of Whole Cell Extracts and Electrophoretic Mobility Shift Assays—Transfected cells were induced with IL-4 (50 ng/ml) for 20 min. They were then washed and supplied with fresh medium. Whole cell extracts were prepared after different time points as described previously (12). A double-stranded oligonucleotide probe containing the STAT6-binding site in the murine Cϵ germ line promoter between positions -117 and -97 was end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs). Unincorporated [γ-^{32}P]ATP was separated from the labeled probe by centrifugation through a MicroSpin™ G-25 column (Amersham Biosciences). The nucleoprotein binding reaction was performed as described previously (32) using 8 μg of whole cell extracts and 20,000 cpm (corresponding to ~0.5 ng) of the labeled probe.

Preparation of RNA and RT-PCR—Total RNA was prepared using Trifast reagent (Peqlab) according to the manufacturer's instructions.

The cDNAs were obtained by reverse transcription of total RNA (2 μg) using Omniscript reverse transcriptase (Qiagen) and random hexamer primers. Amplification of eotaxin-3 cDNA was performed with specific primers (8) and *Taq* polymerase (Invitrogen) according to the manufacturer's instructions. As a control, the cDNA of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified with specific primers (34). For both genes an initial denaturation for 4 min at 94 °C and subsequent 30–35 cycles of PCR were performed. Each cycle consisted of a denaturation period (30 s at 94 °C), an annealing phase (35 s at 60 °C for eotaxin-3 and at 65° for GAPDH), and an extension period (35 s at 72 °C). PCR products were separated by agarose gel electrophoresis, and the DNA was stained with ethidium bromide.

ELISA—To test the affinities of the rabbit antisera to STAT6, a GST 96-well detection module from Amersham Biosciences was used according to the manufacturer's manual. GST-STAT6-TAD was bound to the plates, which were precoated with GST antibody. After washing, rabbit antisera were added and detected using peroxidase-coupled anti-rabbit antibody (Amersham Biosciences). Human eotaxin-3 was measured by ELISA according to a standard streptavidin-horseradish peroxidase assay protocol. Briefly, eotaxin-3 was captured with an anti-human eotaxin-3 antibody and detected with biotinylated anti-human eotaxin-3 antibody (PeproTech) and streptavidin-horseradish peroxidase (Jackson ImmunoResearch Laboratories). As a control, Dulbecco's modified Eagle's medium was analyzed in parallel and used as reference. This value was subtracted from sample values.

RESULTS

A 21-Amino Acid Peptide Contains the STAT6/NCoA-1 Interaction Motif—We have shown recently that the p160/SRC family member NCoA-1 is a specific coactivator of STAT6. STAT6 recruits NCoA-1 via its TAD (15). The NCoA-1-binding site is located at the carboxyl-terminal region between amino acids 792 and 847 of the STAT6-TAD (15). To identify interaction motifs, which mediate the recruitment of NCoA-1, we designed three peptides that cover the NCoA-1-binding site in STAT6-TAD (Fig. 1*A*), and we investigated whether they would act as competitors for the interaction in GST-pulldown experiments. A GST fusion protein containing the TAD of STAT6 was coupled to glutathione-Sepharose and incubated with ^{35}S-labeled NCoA-1 in the presence or absence of the different peptides. As expected, NCoA-1 bound strongly to GST-STAT6-TAD (Fig. 1*B*, *lane 3*) but not to GST (*lane 2*). Peptide 1 efficiently blocked the interaction between STAT6 and NCoA-1 (*lane 4*). In contrast, peptide 2 and peptide 3 had no effect on the binding of STAT6 and NCoA-1 (*lanes 5* and *6*). These results suggest that peptide 1 contains an essential motif for the interaction with NCoA-1 and could therefore act as a competitor for binding.

To determine the concentration of peptide 1 required for efficient inhibition of the STAT6/NCoA-1 binding, a similar experiment was performed with decreasing amounts of peptide 1. For these experiments we used histidine-tagged fusion proteins comprising the STAT6 interaction domain of NCoA-1 defined in our former studies (15) and GST fusion proteins containing the full-length STAT6 sequence. The GST-STAT6 fusion also contains a histidine tag allowing estimation of both protein concentrations by Western blotting. GST or GST-STAT6 were incubated with NCoA-1 (amino acids 213–462) and decreasing amounts of peptide 1. Precipitated proteins were subjected to Western blotting with an antibody recognizing the histidine tag. NCoA-1 bound strongly to GST-STAT6 but not GST alone (Fig. 1*C*, *lanes 2* and *3*). The highest amount of peptide 1 (0.28 mM) completely abolished this interaction (*lane 4*), while at a lower peptide concentration (28 μM) a small amount of bound NCoA-1 could still be detected. The lowest concentration of peptide 1 (2.8 μM, *lane 6*), which represents a 50-fold molar excess over the bound GST-STAT6, was still able to compete for binding of NCoA-1 (compare *lanes 3* and *6*). These results indicate that peptide 1 represents a sequence of STAT6 that is essential for the interaction with NCoA-1 and can therefore act as a competitor in a concentration-dependent manner.

To confirm that the region of STAT6 present in peptide 1 mediates the interaction with NCoA-1, we raised antibodies against the different peptides shown in Fig. 1*A* and tested their ability to block the STAT6/NCoA-1 interaction. Two rabbits (a and b) were immunized with each peptide. We performed GST-pulldown assays with the GST-STAT6-TAD and the radioactively labeled STAT6 interaction domain of NCoA-1 comprising amino acids 213–462 in the presence or absence of antiserum. The binding of GST-STAT6-TAD to NCoA-1 was not affected by the addition of a preimmune serum to the binding reaction (Fig. 1*D*, compare *lane 3* to *4*). Antisera 1a and 1b, which were raised against peptide 1, efficiently blocked the STAT6/NCoA-1 interaction (*lanes 5* and *6*). In contrast, antisera raised against peptides 2 and 3 did not significantly affect the binding (*lanes 7–10*). All antisera had similar binding affinities to STAT6, as determined by ELISA (Fig. 1*E*). The blocking capacity of antisera 1a and 1b indicates that these antibodies cover the specific interaction motif in STAT6, which is then no longer available for NCoA-1. In summary, the experiments suggest that the region of STAT6 spanning amino acids 794–814 harbors a protein interaction motif which mediates the recruitment of NCoA-1.

Mutagenesis of the STAT-LXXLL Motif Abrogates NCoA-1 Binding in Vitro—Sequence analyses of peptide 1 revealed a sequence matching the L*XX*LL consensus sequence (Fig. 2*A*). L*XX*LL motifs mediate protein/protein interactions and were originally found in a variety of coactivators. In order to test whether the L*XX*LL motif of STAT6 is essential for the recruitment of NCoA-1, we mutated the sequence by replacing leucines 802 and 805 with alanine (Fig. 2*A*). GST fusion proteins containing the wild type or mutant TAD of STAT6 (amino acids 642–847) were tested for their binding to NCoA-1 in GST pulldown assays. Equal amounts of the GST fusion proteins or GST alone were incubated with ^{35}S-labeled NCoA-1 (amino acids 213–462). As shown before, wild type GST-STAT6-TAD bound NCoA-1 strongly (Fig. 2*B*, *lane 3*). Point mutations in the L*XX*LL motif completely abolished the binding of GST-STAT6-TAD to NCoA-1 (*lane 4*). Although the two point mutations alter the migration of the STAT6 fusion protein in SDS-PAGE, both wild type and mutant proteins were expressed in similar amounts (Fig. 2*C*, *lane 2* and *3*). This excludes the possibility that the difference in binding was due to a difference in protein input. The results from these experiments clearly demonstrate that the L*XX*LL motif of STAT6 is essential for the interaction with NCoA-1 *in vitro*.

Mutations in the LXXLL Motif Abolish NCoA-1 Recruitment in Vivo—To prove the function of the STAT-L*XX*LL motif in NCoA-1 recruitment *in vivo*, fusions of wild type and mutant STAT-TAD (amino acids 792–847) with the GAL4-DNA binding domain were analyzed in transactivation studies (Fig. 3*A*). 293T cells were transiently transfected with the different GAL4-DNA binding domain fusions, a GAL4 reporter, and the NCoA-1 expression vector or control vector as indicated. Expression of GAL4-DNA binding domain alone did not result in marked expression of the luciferase gene neither in the presence nor in the absence of exogenous NCoA-1 (Fig. 3*B*, *lanes 1* and *2*). The GAL4 DNA binding domain fusion with wild type STAT6 amino acids 792–847 strongly enhanced luciferase expression (*lane 3*). This induction was increased 2-fold by coexpression of NCoA-1 (*lane 4*). The mutant GAL4 STAT6 fusion displayed only 20% of the wild type transactivation potential (compare *lanes 5* and *3*). Furthermore, NCoA-1 could not stimulate the transactivation potential of the mutant GAL4-STAT6 fusion (*lane 6*). To verify these results, we repeated the exper-

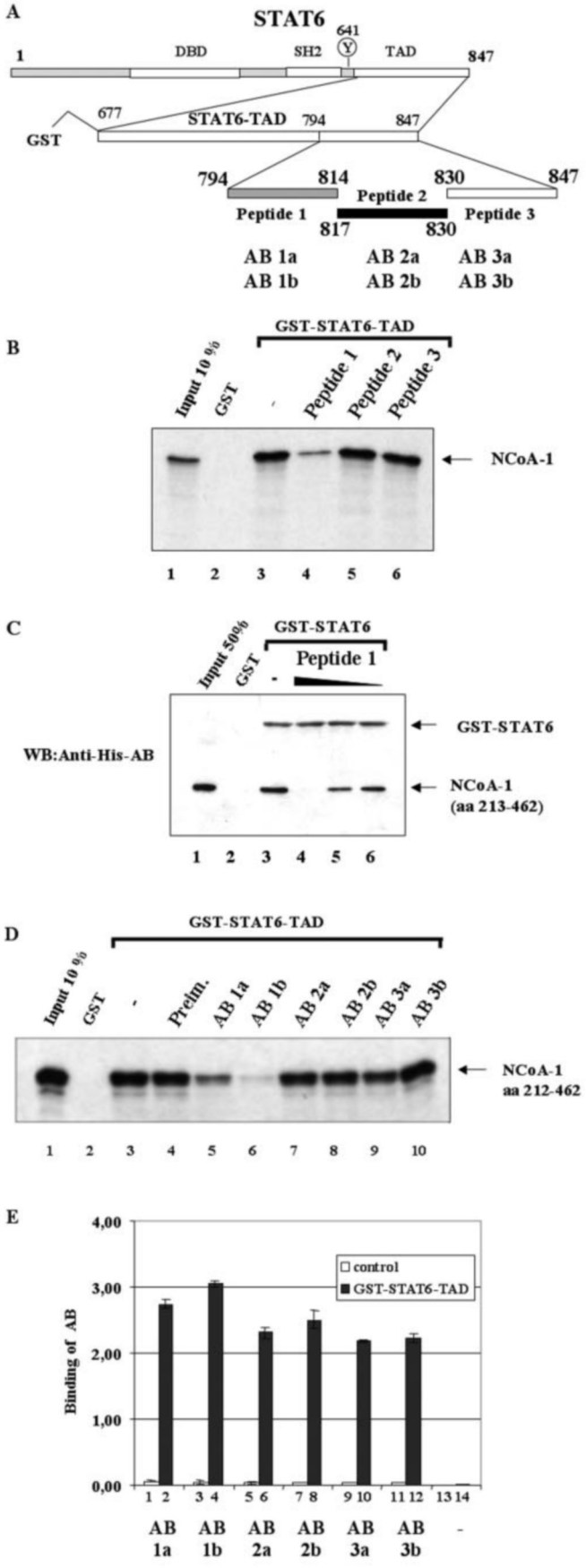

FIG. 1. **Peptide 1 is a potent inhibitor of the interaction between STAT6 and NCoA-1 *in vitro*.** *A*, the structure of human STAT6, the GST-STAT-TAD fusion protein, and peptides derived from the TAD and antisera raised against the different peptides. Amino acid position, DBD, Src homology 2 (*SH2*) domain, TAD, and the cytokine-dependent phosphorylation site (*Y*) are indicated. *B*, GST or the GST-STAT6-TAD fusion proteins were purified from *E. coli*, bound to glutathione-Sepharose, and incubated with the ^{35}S-labeled NCoA-1 in the presence of 100 μg of peptides as indicated. Material bound to the glutathione-Sepharose was recovered from the binding reaction, extensively washed, and analyzed by SDS-PAGE and fluorography (*lanes 2–6*). Input control in *lane 1* reflects 10% of the total amount of ^{35}S-labeled protein used in the experiments. *C*, GST-STAT6 was purified from Sf9 insect cells infected with baculovirus expression vector for GST and histidine-tagged full-length STAT6. GST or GST-STAT6 was bound to glutathione-Sepharose and incubated with the histidine-tagged NCoA-1 fusion protein comprising residues 213–462, purified from *E. coli*. Binding was carried out in the presence of 0.28 mM (*lane 4*), 28 μM (*lane 5*), and 2.8 μM (*lane 6*) peptide 1 as indicated. Precipitated proteins were washed, eluted, and resolved by SDS-PAGE. An aliquot of the NCoA-1 fragment corresponding to 50% of the material used for the interaction assay was analyzed in parallel (*lane 1*). Histidine fusion proteins were visualized by Western blot analysis with an antibody recognizing the histidine tag. *D*, GST or GST-STAT6-TAD fusion protein, bound to glutathione-Sepharose, was incubated with ^{35}S-labeled histidine-NCoA-1 fusion protein, comprising residues 213–462. In samples 4–8 the binding reaction was carried out in the presence of 10 μl of the different rabbit antisera (*AB 1a*, *AB 1b*, *AB 2a*, *AB 2b*, *AB 3a*, and *AB 3b*) or preimmune sera as indicated. Bound proteins were analyzed by SDS-PAGE and fluorography (*lanes 2–8*). As a control, 10% of the ^{35}S-labeled protein used in the experiments was analyzed in parallel (*lane 1*). *E*, the rabbit sera were analyzed for binding to STAT6 in ELISA. GST-STAT6-TAD fusion protein was immobilized on plates that were coated with GST antibodies. The rabbit antisera were added to the wells containing GST-STAT6-TAD (*black bars*) or buffer as a control (*white bars*). Bound antibodies were detected using peroxidase-coupled anti-rabbit antibody. As a control, the binding of secondary antibody to the plates was analyzed in parallel (*lanes 13* and *14*). aa, amino acids.

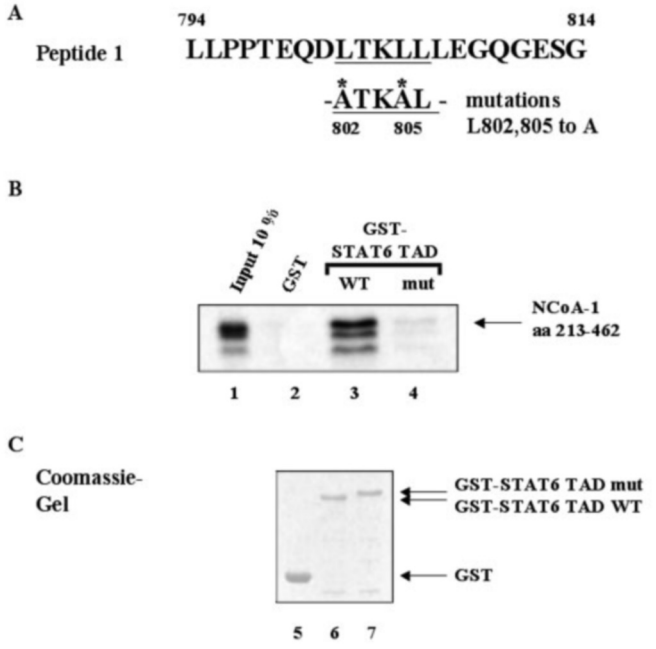

FIG. 2. **Point mutations in the L*XX*LL motif of STAT6-TAD abolish the interaction with NCoA-1 *in vitro*.** *A*, the sequences of peptide 1 and the L*XX*LL motif. Alanine mutations introduced into the sequence are labeled with *asterisks*. *B*, GST or GST fusion proteins containing residues 642–847 of wild type (*WT*) or mutant (*mut*) STAT6 were incubated with ^{35}S-labeled NCoA-1 fragment, comprising residues 213–462. Material bound to glutathione-Sepharose was separated by SDS-PAGE. Radiolabeled protein was detected by fluorography. *C*, a quarter of each of the reactions was separately analyzed by SDS-PAGE and Coomassie staining in order to detect the GST fusion proteins.

iments in the lung epithelial cell line NCI-H292. As observed in 293T cells basal transactivation by the GAL4-DNA binding domain alone was not affected by coexpression of NCoA-1 (Fig. 3*C*, *lanes 1* and *2*). In contrast to what we observed in 293T

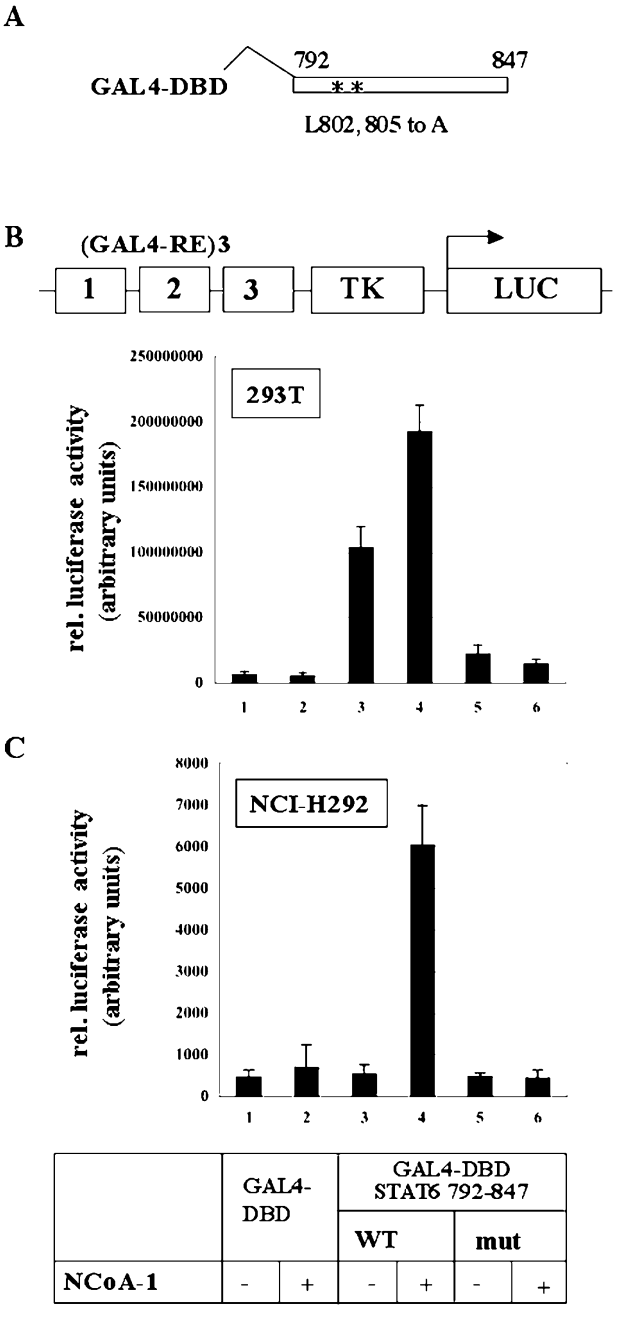

FIG. 3. **Point mutations in the L*XX*LL motif of STAT6-TAD abolish the recruitment of NCoA-1 in cells.** *A*, schematic representation of the fusion protein containing the GAL4-DNA-DBD and the carboxyl-terminal transactivation domain of STAT6. The GAL4-DNA binding domain DBD (amino acids 1–147) was fused to residues 792–847 of STAT6. The position of point mutations is indicated by an *asterisk*. *B*, reporter plasmid (*GAL4-RE)3 TK LUC* (0.5 μg), expression plasmid encoding GAL4-DBD-STAT6 fusion proteins (25 ng), NCoA-1, or empty expression vector (100 ng), and SV40-*lacZ* (50 ng) were transfected into 293T cells as indicated. *A*, NCI-H292 cells were transfected with the reporter plasmid (*GAL4-RE)3 TK LUC* (0.5 μg), expression plasmid encoding GAL4-DBD-STAT6 fusion proteins (100 ng), NCoA-1, or empty expression vector (200 ng) and SV40-*lacZ* (100 ng), as indicated. *B* and *C*, luciferase activities were determined and normalized against β-galactosidase activities. The average values with standard deviations of three independent experiments are shown. *WT*, wild type; *mut*, mutant.

cells, the wild type GAL4-STAT6 fusion did not activate transcription when transfected alone indicating that NCoA-1 is more limiting in this cell line (*lane 3*). Cotransfection of NCoA-1 dramatically stimulated the transactivation potential of wild type GAL4-STAT6 (*lane 4*). In contrast, NCoA-1 could not enhance transactivation by the mutant GAL4-STAT6 fusion (*lane 6*). Together these data support the proposal that the L*XX*LL motif of STAT6 is required for recruitment of NCoA-1 *in vivo* and for NCoA-1-mediated transcriptional activation.

The LXXLL Motif of STAT6 Is Required for Full Transactivation but Is Not Involved in DNA Binding—In order to study the function of the L*XX*LL motif in IL-4-mediated transactivation, we introduced the L802A,L805A mutation into the full-length STAT6 sequence (Fig. 4*A*).

We first investigated whether the mutation would disrupt recruitment of NCoA-1 by full-length STAT6 protein. Coimmunoprecipitation experiments were performed with both STAT6 variants and NCoA-1. We used GFP-tagged STAT6 versions, which can be efficiently immunoprecipitated by anti-GFP serum. 293T cells were transfected with expression vectors for the different STAT6 variants together with NCoA-1. Both STAT6 proteins were immunoprecipitated from cell lysates. The presence of NCoA-1 in the protein complexes was analyzed by Western blot. NCoA-1 was coimmunoprecipitated by wild type STAT6 protein (Fig. 4*B*, *lane 6*) but not by the mutant STAT6 (*lane 8*). Western blot performed with STAT6-specific antibodies indicated that there were comparable amounts of both STAT6 proteins precipitated (*lanes 2* and *4*). These data show that the mutant STAT6 version is not at all capable of forming a complex with NCoA-1 in cells.

Next we tested whether such a mutation would affect the activation or the DNA-binding ability of STAT6. 293T cells were reported to contain a defective STAT6 (11, 35). We therefore used this cell line to study the function of the STAT6-L802A,L805A mutant without interference from endogenous STAT6. Wild type and mutant STAT6 were transfected into 293T cells, and DNA binding activity was analyzed by electrophoretic mobility shift assays with cell lysates. To determine the DNA binding stability of wild type and the STAT6-L802A,L805A variant cells were treated with IL-4 for 20 min, after which the medium was replaced with cytokine-free medium. Cells were harvested at various time points thereafter. Extracts from mock-transfected cells showed no STAT6-DNA complex confirming that the endogenous defective STAT6 cannot bind to DNA (Fig. 4*C*, *lanes 1–4*). Both wild type and the STAT6 variant specifically bound to the probe, and this binding did not decrease even after 2 h (*lane 5–12*). No difference in complex formation was observed between wild type and the STAT6 variant indicating that the L802A,L805A mutations do not influence activation by tyrosine phosphorylation or DNA binding of STAT6.

To evaluate the transactivation function of the STAT6 variant, 293T cells were transfected with either wild type or variant STAT6 together with a luciferase reporter construct driven by multimerized STAT6-response elements. Luciferase expression was investigated in cell lysates after stimulation with IL-4. As expected, endogenous STAT6 was not able to induce luciferase expression of the reporter plasmid (Fig. 4*D*, *lanes 1* and *2*). Transfection of wild type STAT6 strongly enhanced the reporter activity after IL-4 stimulation (*lane 4*), whereas the STAT6 variant displayed only 50% of the wild type activity (*lane 6*). Similar amounts of wild type and variant STAT6 were expressed in the transfected cells as proven by Western blotting (Fig. 4*E*, compare *lanes 2* and *3*). This rules out the possibility that the distinct transactivation potentials result from a difference in STAT6 variant expression. Instead these results clearly show that the L*XX*LL motif is required for full STAT6-mediated transactivation and indicate that NCoA-1 recruitment by this motif mediates 50% of the transactivation potential of STAT6.

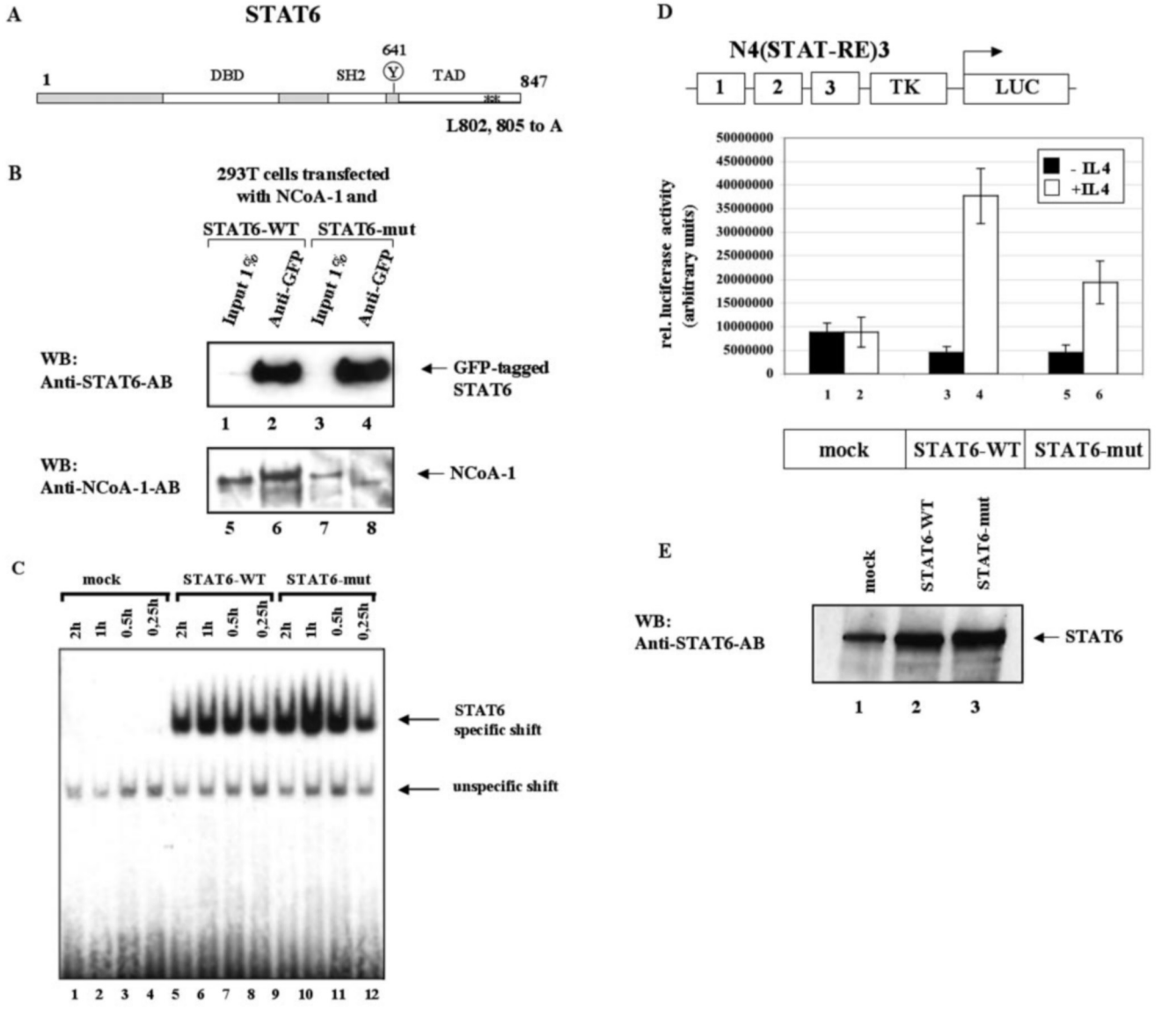

FIG. 4. **Point mutations in the L*XX*LL motif of STAT6 do not affect DNA binding but decrease the transactivation potential of STAT6.** *A*, schematic representation of functional domains of STAT6. Depicted are DBD, Src homology 2 (*SH2*) domain, TAD, and the cytokine-dependent phosphorylation site (*Y*). *Asterisks* indicate the position of point mutations. *B*, 293T cells were transfected with 3 μg of expression vector for GFP-tagged wild type or mutant STAT6 together with NCoA-1 expression vector. STAT6 proteins were immunoprecipitated from cell lysates using anti-GFP serum. Immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting (*WB*). 1% of cell lysates was analyzed in parallel (*lanes 1*, *3*, *5*, and *7*). STAT6 (*upper panel*) and NCoA-1 (*lower panel*) were detected with specific antibodies. *C*, 293T cells were transfected with 2 μg of empty expression vector (*lanes 1–4*) or expression vectors for STAT6 (*lanes 5–8*) or STAT6-mut (*lanes 9–12*) and treated with IL-4 for 20 min. Cytokine was removed from the cells, and whole cell extracts were prepared after different time points as indicated. Equal amounts of extracts were incubated with a radiolabeled double-stranded oligonucleotide probe containing the $-117/-97$ segment of the murine Cϵ germ line promoter. Nucleoprotein complexes were resolved by PAGE and visualized by autoradiography. *D*, 293T cells were transfected with N4(STAT-RE)3 LUC (0,36 μg) reporter plasmid, expression vectors for STAT6, STAT6-mut, or empty expression vector (100 ng), and SV40-*lacZ* expression plasmid (50 ng) as indicated. Cells were treated with IL-4 or left untreated. Luciferase activities were determined in cell lysates and normalized against β-galactosidase activities. The values represent the average from three independent experiments. *E*, cell lysates from C were analyzed by Western blot (*WB*) using STAT6-specific antiserum.

The STAT6-LXXLL Motif Contributes to IL-4-regulated Eotaxin-3 Expression—Because transcriptional activity of a reporter gene driven by multimerized binding sites does not really reflect the transcriptional regulation of chromatin-packaged genes, the contribution of the STAT6-L*XX*LL motif for transactivation of an endogenous target gene was analyzed. 293T cells have been described to express the chemokine eotaxin-3 in an IL-4-dependent manner only after transfection with functional STAT6. Thus we determined eotaxin-3 expression in 293T cells transiently transfected with either wild type or mutant STAT6 after IL-4 stimulation (Fig. 5). In accordance with a previous report (35), we could detect IL-4-enhanced eotaxin-3 mRNA expression by RT-PCR only after transfection of STAT6 (Fig. 5*A*, *lane 4*). Transfection of the STAT6-L802A,L805A variant also resulted in eotaxin-3 expression in IL-4-treated cells but to a lower extent (compare *lanes 4* and *6*). To evaluate the difference in eotaxin-3 induction by wild type and mutant STAT6 on the protein level, secreted eotaxin-3 was measured in cell culture supernatants by ELISA. As expected from the RT-PCR results, mock-transfected cells did not secrete detectable levels of eotaxin-3, even after cytokine treatment (Fig. 5*B*, *lanes 1* and *2*), whereas cells transfected with wild type STAT6 produced high amounts of eotaxin-3 upon cytokine treatment (*lane 4*). Transfection of the STAT6-L802A,L805A

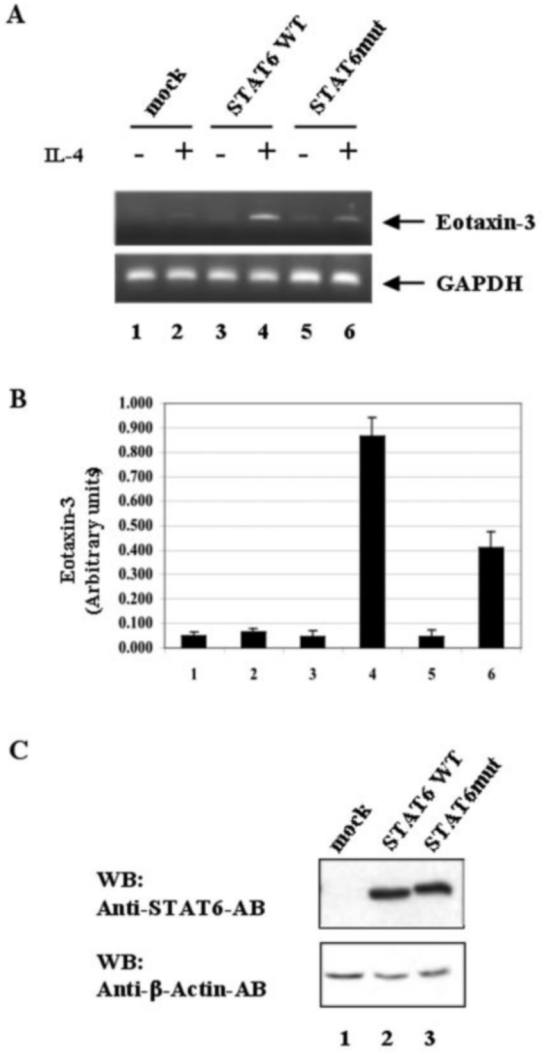

FIG. 5. **Point mutations in the L*XX*LL motif decrease expression of eotaxin-3 in 293T cells.** *A*, 293T cells were transfected with expression vectors encoding wild type (*WT*) or mutant STAT6 or empty expression vector (2 μg). Cells were induced with IL-4 for 16 h or left untreated as indicated. Total RNA was prepared, and the expression level of eotaxin-3 and GAPDH was analyzed by RT-PCR. *B*, cells transfected as described in *A* were induced with IL-4 for 48 h or left untreated. Secreted eotaxin-3 was detected in cell culture supernatants by ELISA. The average values with standard deviations of three independent experiments are shown. *C*, cells from *B* were lysed and analyzed by SDS-PAGE and Western blot (*WB*) using antibodies against STAT6 and β-actin.

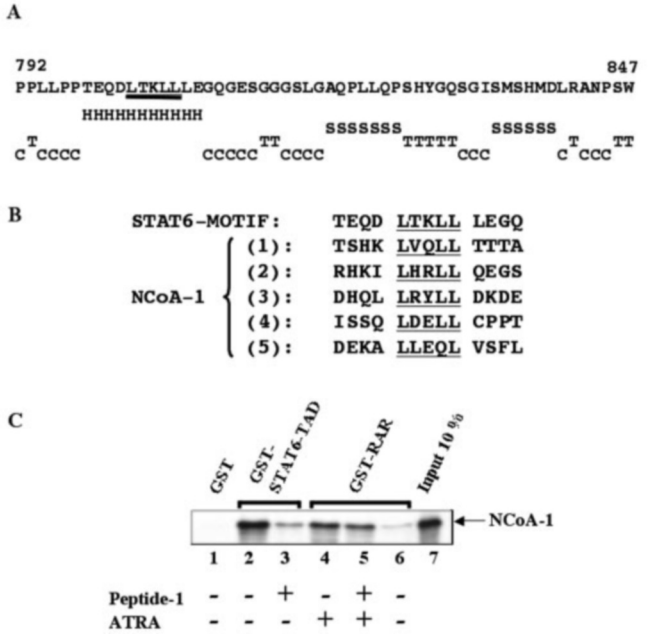

FIG. 6. **Peptide 1 specifically inhibits the L*XX*LL-dependent STAT6/NCoA-1 interaction but not RAR/NCoA-1 interaction.** *A*, the secondary structure of the L*XX*LL motif containing the sequence of STAT6 represented by peptide 1 was predicted by DNASIS software. Likely *H* (helical), *S* (β-sheet), *T* (turn), and *C* (coiled) structures are shown. *B*, sequence alignment of L*XX*LL motif of STAT6 and from motifs derived from murine NCoA-1. Motifs 1–3 from NCoA-1 reside in the nuclear receptor interaction domain, and motifs 4 and 5 are derived from the CBP interaction domain. The core L*XX*LL motifs are *underlined*. *C*, GST, GST-STAT6-TAD or the GST-retinoic acid receptor α (*RAR*) fusion protein were purified from *E. coli*, bound to glutathione-Sepharose, and incubated with ^{35}S-labeled NCoA-1. To activate RAR, ligand was added (10^{-6} M all-*trans*-retinoic acid (*ATRA*)) (*lanes 4* and *5*). In samples 3 and 5 the binding reaction was carried out in the presence of 50 μg of peptide 1. Bound proteins were analyzed by SDS-PAGE and fluorography (*lanes 2–8*). As a control, 10% of the ^{35}S-labeled protein used in the experiments was analyzed in parallel (*lane 7*).

variant also enabled cells to produce eotaxin-3 but again at a reduced level. Only 50% of wild type expression was achieved (compare *lanes 4* and *6*). To confirm that the difference in eotaxin-3 expression was not due to a variance in STAT6 expression level, Western blot analysis was performed with cell lysates. As shown in Fig. 5*C*, both wild type and variant STAT6 were expressed in similar amounts. These data correspond nicely to the results obtained from transfection experiments with an artificial STAT6 reporter gene confirming that the STAT6-mediated transactivation is impaired when the L*XX*LL motif is mutated. The L*XX*LL motif hence contributes substantially to STAT6-mediated transactivation.

Peptide 1 Comprising the STAT-LXXLL Motif Does Not Influence RAR/NCoA-1 Interaction—The functional characterization of the STAT6-L*XX*LL suggests that it is the most important motif for the interaction with NCoA-1. Thus we wondered whether this motif would be structurally and functionally related to the L*XX*LL motifs that are present in NCoA-1 itself. These motifs form an α-helical structure with a hydrophobic face built by the conserved leucines. To obtain initial hints about the structure, we performed a protein two-dimensional structure prediction of the sequence spanning the L*XX*LL motif using DNASIS software, as depicted in Fig. 6*A*. This indicated that the L*XX*LL motif of STAT6 most likely forms an α-helical structure as well. A sequence alignment with the L*XX*LL motifs of NCoA-1 revealed that the STAT6-L*XX*LL motif does not share any obvious homology to the core motif or adjacent residues (Fig. 6*B*). To prove the unique structure and function of the STAT6-L*XX*LL motif, we investigated whether inhibitory peptide 1 described in Fig. 1, which contains the STAT6-L*XX*LL motif, would affect the L*XX*LL-based interaction of NCoA-1 with nuclear hormone receptors. We performed a binding assay with the retinoic acid receptor α (RAR) and NCoA-1, because this interaction has been described previously to be dependent on two L*XX*LL motifs of NCoA-1 (30). GST-RAR bound strongly to radioactively labeled NCoA-1 in the presence (Fig. 6*C*, *lane 4*) but not in the absence (*lane 6*) of all-*trans*-retinoic acid. Addition of peptide 1 to the binding reaction did not affect the recruitment of NCoA-1 to GST-RAR (*lane 5*). However, binding of GST-STAT6-TAD to NCoA-1 was completely blocked in the presence of peptide 1 (compare *lanes 2* and *3*). Peptide 1 was also not functional in blocking the NCoA-1/estrogen receptor α interaction (data not shown). Hence, peptide 1 acts as a specific inhibitor of the STAT6/NCoA-1 interaction and cannot compete with the L*XX*LL motifs of NCoA-1 for nuclear receptor binding. These data confirm

that the STAT6-L*XX*LL motif is not related to the NCoA-1-L*XX*LL motifs.

DISCUSSION

The aim of this study was the identification of essential motifs in the transactivation domain of STAT6 to get new insights into the transactivation mechanism of STAT6 and to identify targets to inhibit the STAT6 transactivation function. Transcriptional activation requires the recruitment of coactivators by DNA-bound transcription factors. This often occurs via protein/protein interaction mediated by distinct structural motifs within the transactivation domains of the DNA-bound transcription factors and the coactivator interaction domains (36). In previous studies (14, 15) we have shown that at least two coactivators, p300/CBP and NCoA-1, are involved in STAT6-mediated transactivation. Both coactivators bind independently to different parts of the STAT6 transactivation domain. NCoA-1 binds between residues 792 and 847 of the STAT6-TAD.

In this study we have further characterized this interaction domain. Peptides spanning different parts of the STAT6 interaction domain and antibodies raised against these peptides were used to find potential interaction motifs. A peptide representing the first part of the STAT6 interaction domain (amino acids 794–814) efficiently competed for NCoA-1 binding. Moreover, antibodies recognizing this region in STAT6 efficiently blocked NCoA-1 binding, indicating that the NCoA-1 interaction motif lies between amino acids 794 and 814 of STAT6. Importantly, antibodies that bind very closely adjacent to the STAT6-binding motif were not able to block NCoA-1 binding, confirming that a particular motif has to be masked to prevent NCoA-1 binding. These experiments showed that it is possible to target the STAT6/NCoA-1 interaction very specifically either by masking the binding domain of NCoA-1 with peptides representing the STAT6-binding motif or by covering the STAT6-binding motif with an antibody. The inhibitory peptides and antibodies identified here will be valuable tools for further *in vivo* studies, *e.g.* microinjection experiments to investigate whether blocking the NCoA-1 recruitment will inhibit STAT6-mediated transactivation. In addition, the inhibitory peptide can present the starting point for the development of small molecules that block the STAT6/NCoA-1 interaction.

The sequence of peptide 1 contains an L*XX*LL motif (where L is a leucine and *X* any amino acid). This motif was originally identified in transcriptional coactivators and mediates the interaction of these proteins with nuclear hormone receptors (31). L*XX*LL motifs are necessary and sufficient for the binding of these coactivators to ligand-bound nuclear hormone receptors, and they are required for coactivation of transcription. Here we demonstrate that the L*XX*LL motif in STAT6 is involved in the interaction of STAT6 with its coactivator NCoA-1. Point mutations (L802A,L805A) in the L*XX*LL motif (Fig. 2*A*) completely abolished the interaction of GST-STAT6-TAD to NCoA-1 (Fig. 2*B*), demonstrating the crucial role of the L*XX*LL motif for binding *in vitro*. Furthermore, we observed that the STAT6-L*XX*LL motif is absolutely required to recruit NCoA-1 to the promoter and to enhance transcription by the GAL4-DNA binding domain fusion of STAT6-TAD (amino acids 792–847). The transactivation potential of this GAL4 fusion seems to be based entirely on NCoA-1, which is present at different levels in 293T and NCI-H292 cells (data not shown). In 293T cells, which express high NCoA-1 levels, GAL4-STAT6-TAD strongly enhanced transcription (Fig. 3*B*). In NCI-H292 cells, which express a much lower level, NCoA-1 seemed to be a limiting factor, leading to a weak transactivation potential (Fig. 3*C*). Cotransfection of NCoA-1 dramatically amplified transactivation by GAL4-STAT6-TAD. Recruitment of NCoA-1 to the GAL4-STAT6-TAD was strictly dependent on the intact L*XX*LL motif, because mutations of the motif completely abolished the transactivation potential of the GAL4-STAT6-TAD fusion in both cell lines, and this could not be rescued by coexpression of NCoA-1 (Fig. 3, *B* and *C*).

We analyzed the function of the L*XX*LL motif in transcriptional activation by full-length STAT6. These experiments were performed in 293T cells, which lack functional STAT6 and can therefore be used for analyzing the function of STAT6 variants. In addition, the ability of 293T cells to produce eotaxin-3 after IL-4 treatment allowed us to analyze the contribution of the STAT6-L*XX*LL motif to the transactivation of an endogenous target gene (8).

The L802A,L805A mutation did not affect the activation by tyrosine phosphorylation or the DNA binding of STAT6 after IL-4 treatment (Fig. 4*B*). However, the ability to activate transcription on an artificial reporter gene containing multimerized STAT6-binding sites (Fig. 4) as well as on the endogenous eotaxin-3 target gene (Fig. 5) was strongly reduced. The STAT6- L802A,L805A variant displayed in both assays a 50% reduced transactivation potential compared with wild type STAT6. Significantly, the finding that eotaxin-3 protein expression was also reduced by 50% in STAT6 variant-expressing cells confirmed that the recruitment of NCoA-1 by the STAT6-L*XX*LL motif is an essential step in the transcriptional activation of chromatin-packaged endogenous genes. IL-4-induced transcriptional activation of the eotaxin-3 promoter is highly dependent on STAT6-binding sites (8). In contrast to other STAT6 target genes, *e.g.* the Cϵ germ line gene, no activation or synergistic effects on transcription by NF-κB was detected. Our results so far demonstrate that the expression of eotaxin-3 is largely dependent on the recruitment of NCoA-1 by the STAT6-L*XX*LL motif. However, we could not predict that this is true for all IL-4-regulated genes, especially when they are already activated by tumor necrosis factor-α. It is possible that the role of NCoA-1 in STAT6-mediated transactivation varies depending on the specific promoter context as well as on the cellular context. Further studies have to be performed to investigate the importance of the L*XX*LL motif for other STAT6 target genes.

In previous studies we observed that the coactivator p300/CBP, which associates with NCoA-1 (19, 20), binds directly to a different region of the STAT6-TAD (amino acids 677–791) (15). Therefore NCoA-1 could still be recruited to the STAT6-L*XX*LL mutant via bridging to p300/CBP. In this case NCoA-1 would have an even greater role in STAT6 transactivation. To evaluate the exact contribution of NCoA-1 for STAT6 transactivation, the transactivation potential of STAT6 has to be determined in cells lacking this coactivator.

L*XX*LL motifs are abundant in different coactivators. Many coactivators contain several L*XX*LL motifs. The p160/SRC family members contain three motifs, which mediate the interaction with the nuclear hormone receptors and two motifs, which mediate the p300/CBP interaction. p300/CBP itself contains two motifs (31). All motifs described so far form α-helical structures with a hydrophobic face, which is built by the conserved leucines. However, not all L*XX*LL motifs are functionally equivalent. Sequences flanking the L*XX*LL motif determine target specificity (37, 38). We analyzed whether the STAT6-L*XX*LL motif has any structural or functional relation to the NCoA-1-L*XX*LL motifs. From the structural prediction we assumed that the STAT6-L*XX*LL motif also forms an α-helix. However, this motif does not share any homologous residues amino- or carboxyl-terminally to the L*XX*LL core sequence with the motifs in the p160/SRC or p300/CBP coactivators (Fig. 6 and data not shown). Because positive as well as negative

cross-talk between STAT proteins and nuclear receptors has been described (39, 40), we tested whether the STAT6-L*XX*LL motif could compete with the NCoA-1-L*XX*LL motif for nuclear receptor binding. We demonstrated that peptide 1, which represents the STAT6-L*XX*LL motif and which was a potent inhibitor of the STAT6/NCoA-1 interaction, was not able to inhibit the NCoA-1/retinoic acid receptor α (Fig. 6) or NCoA-1/estrogen receptor interaction (data not shown). This suggests that the STAT6-L*XX*LL motif is unique and has most likely no function in cross-talk. It can also be expected that the structure of the STAT6-L*XX*LL is unique. Structural analyses of the STAT6-L*XX*LL peptide in complex with the STAT6 binding domain of NCoA-1, which we mapped to a region between amino acids 213 and 462 (15), are currently in progress.

The IL-4/STAT6 signaling pathway is an attractive target for the treatment of allergic diseases, because STAT6 is crucial for class switching to IgE, a pivotal factor in the pathogenesis of asthma. Several approaches have been made to inhibit this pathway as follows: oligonucleotides, which specifically inhibit binding of STAT6 to its response element (41, 42); intracellular peptides, which inhibit the binding of STAT6 to the receptor (43); antagonistic IL-4 mutants (44); and IL-4-receptor antagonists (45). We revealed here a novel approach to inhibit this pathway. The STAT6-L*XX*LL motif will be a new, attractive drug target. This motif is required for the STAT6-mediated expression of eotaxin-3, a potent chemoattractant of eosinophils. The recruitment of eosinophils to sites of inflammation is a hallmark of allergic diseases. Therefore inhibition of eotaxin-3 would be a beneficial therapy. It might be feasible to inhibit the STAT6 signaling pathway by targeting the STAT6-L*XX*LL motif without affecting similar motifs. Moreover, small drug inhibitors can be developed based on the peptide characterized here that will be able to inhibit the STAT6/NCoA-1 interaction.

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REFERENCES

1. Darnell, J. E., Kerr, I. M., and Stark, G. R. (1994) *Science* **264,** 1415–1421
2. Darnell, J. E. (1997) *Science* **277,** 1630–1635
3. Ihle, J. N. (1996) *Cell* **84,** 331–334
4. Igaz, P., Toth, S., and Falus, A. (2001) *Inflamm. Res.* **50,** 435–441
5. Shimoda, K., van Deursen, J., Sangster, M. Y., Sarawar, S. R., Carson, R. T., Tripp, R. A., Chu, C., Quelle, F. W., Nosaka, T., Vignali, D. A., Doherty, P. C., Grosveld, G., Paul, W. E., and Ihle, J. N. (1996) *Nature* **380,** 630–633
6. Kaplan, M. H., Schindler, U., Smiley, S. T., and Grusby, M. J. (1996) *Immunity* **4,** 313–319
7. Shinkai, A., Yoshisue, H., Koike, M., Shoji, E., Nakagawa, S., Saito, A., Takeda, T., Imabeppu, S., Kato, Y., Hanai, N., Anazawa, H., Kuga, T., and Nishi, T. (1999) *J. Immunol.* **163,** 1602–1610
8. Hoeck, J., and Woisetschlager, M. (2001) *J. Immunol.* **167,** 3216–3222
9. Hampsey, M., and Reinberg, D. (1999) *Curr. Opin. Genet. & Dev.* **9,** 132–139
10. Chen, H., Tini, M., and Evans, R. M. (2001) *Curr. Opin. Cell Biol.* **13,** 218–224
11. Lu, B., Reichel, M., Fisher, D. A., Smith, J. F., and Rothman, P. (1997) *J. Immunol.* **159,** 1255–1264
12. Moriggl, R., Berchtold, S., Friedrich, K., Standke, G. J., Kammer, W., Heim, M., Wissler, M., Stocklin, E., Gouilleux, F., and Groner, B. (1997) *Mol. Cell. Biol.* **17,** 3663–3678
13. Goenka, S., Youn, J., Dzurek, L. M., Schindler, U., Yu-Lee, L. Y., and Boothby, M. (1999) *J. Immunol.* **163,** 4663–4672
14. Gingras, S., Simard, J., Groner, B., and Pfitzner, E. (1999) *Nucleic Acids Res.* **27,** 2722–2729
15. Litterst, C. M., and Pfitzner, E. (2001) *J. Biol. Chem.* **276,** 45713–45721
16. Goodman, R. H., and Smolik, S. (2000) *Genes Dev.* **14,** 1553–1577
17. Vo, N., and Goodman, R. H. (2001) *J. Biol. Chem.* **276,** 13505–13508
18. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382,** 319–324
19. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) *Cell* **85,** 403–414
20. Yao, T. P., Ku, G., Zhou, N., Scully, R., and Livingston, D. M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 10626–10631
21. Xu, L., Glass, C. K., and Rosenfeld, M. G. (1999) *Curr. Opin. Genet. & Dev.* **9,** 140–147
22. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1995) *Science* **270,** 1354–1357
23. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. (1996) *EMBO J.* **15,** 3667–3675
24. Hong, H., Kohli, K., Garabedian, M. J., and Stallcup, M. R. (1997) *Mol. Cell. Biol.* **17,** 2735–2744
25. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) *Nature* **387,** 677–684
26. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y., and Evans, R. M. (1997) *Cell* **90,** 569–580
27. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. (1997) *Science* **277,** 965–968
28. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) *Science* **279,** 703–707
29. Kurokawa, R., Kalafus, D., Ogliastro, M. H., Kioussi, C., Xu, L., Torchia, J., Rosenfeld, M. G., and Glass, C. K. (1998) *Science* **279,** 700–703
30. McInerney, E. M., Rose, D. W., Flynn, S. E., Westin, S., Mullen, T. M., Krones, A., Inostroza, J., Torchia, J., Nolte, R. T., Assa-Munt, N., Milburn, M. V., Glass, C. K., and Rosenfeld, M. G. (1998) *Genes Dev.* **12,** 3357–3368
31. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) *Nature* **387,** 733–736
32. Pfitzner, E., Kirfel, J., Becker, P., Rolke, A., and Schule, R. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5539–5544
33. Pfitzner, E., Jahne, R., Wissler, M., Stoecklin, E., and Groner, B. (1998) *Mol. Endocrinol.* **12,** 1582–1593
34. Worm, M., Krah, J. M., Manz, R. A., and Henz, B. M. (1998) *Blood* **92,** 1713–1720
35. Hoeck, J., and Woisetschlager, M. (2001) *J. Immunol.* **166,** 4507–4515
36. Chen, L. (1999) *Curr. Opin. Struct. Biol.* **9,** 48–55
37. Chang, C., Norris, J. D., Gron, H., Paige, L. A., Hamilton, P. T., Kenan, D. J., Fowlkes, D., and McDonnell, D. P. (1999) *Mol. Cell. Biol.* **19,** 8226–8239
38. Ding, X. F., Anderson, C. M., Ma, H., Hong, H., Uht, R. M., Kushner, P. J., and Stallcup, M. R. (1998) *Mol. Endocrinol.* **12,** 302–313
39. Stocklin, E., Wissler, M., Gouilleux, F., and Groner, B. (1996) *Nature* **383,** 726–728
40. Biola, A., Andreau, K., David, M., Sturm, M., Haake, M., Bertoglio, J., and Pallardy, M. (2000) *FEBS Lett.* **487,** 229–233
41. Wang, L. H., Yang, X. Y., Kirken, R. A., Resau, J. H., and Farrar, W. L. (2000) *Blood* **95,** 1249–1257
42. Stutz, A. M., Hoeck, J., Natt, F., Cuenoud, B., and Woisetschlager, M. (2001) *J. Biol. Chem.* **276,** 11759–11765
43. Stolzenberger, S., Haake, M., and Duschl, A. (2001) *Eur. J. Biochem.* **268,** 4809–4814
44. Grunewald, S. M., Werthmann, A., Schnarr, B., Klein, C. E., Brocker, E. B., Mohrs, M., Brombacher, F., Sebald, W., and Duschl, A. (1998) *J. Immunol.* **160,** 4004–4009
45. Tomkinson, A., Duez, C., Cieslewicz, G., Pratt, J. C., Joetham, A., Shanafelt, M. C., Gundel, R., and Gelfand, E. W. (2001) *J. Immunol.* **166,** 5792–5800