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Immunology

The granulocyte–macrophage colony-stimulating factor/interleukin 3 locus is regulated by an inducible cyclosporin A-sensitive enhancer

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ABSTRACT **Granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin 3 (IL-3) are pleiotropic hemopoietic growth factors whose genes are closely linked and induced in T lymphocytes in a cyclosporin A (CsA)-sensitive fashion. Since we found that the human GM-CSF and IL-3 proximal promoters were not sufficient to account for the observed regulation of these genes, we mapped DNase I hypersensitive sites across the GM-CSF/IL-3 locus in the Jurkat human T-cell line to identify additional regulatory elements. We located an inducible DNase I hypersensitive site, 3 kb upstream of the GM-CSF gene, that functioned as a strong CsA-sensitive enhancer of both the GM-CSF and IL-3 promoters. Binding studies employing Jurkat cell nuclear extracts indicated that four sites within the enhancer associate with the inducible transcription factor AP1. Three of these AP1 elements lie within sequences that also associate with factors resembling the CsA-sensitive, T cell-specific transcription factor NFAT. We provide additional evidence suggesting that an AP1-like factor represents one of the components of NFAT. We propose that the intergenic enhancer described here is required for the correctly regulated activation of both GM-CSF and IL-3 gene expression in T cells and that it mediates the CsA sensitivity of the GM-CSF/IL-3 locus.**

Granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin 3 (IL-3) are tightly regulated cytokines that direct the proliferation, differentiation, and function of a variety of hemopoietic cells and their precursors (1). Their genes are closely linked; they are just 10 kb apart on human chromosome 5 (2). Both genes are expressed in most T lymphocytes and T-cell lines following activation of the T-cell antigen receptor and other surface molecules (1).

The T-cell-receptor-dependent induction of GM-CSF, IL-3, and several other cytokine genes can be blocked by the powerful immunosuppressant cyclosporin A (CsA) (3, 4). The mechanism of action of CsA is well documented only for the interleukin 2 (IL-2) gene, which has a promoter that is strongly induced during T-cell activation (5–9). CsA blocks signals transmitted by the T-cell receptor, which mediate the induction of specific transcription factors such as NFAT that associate with the IL-2 promoter. NFAT is a T cell-specific CsA-inhibitable transcription factor induced early in the course of T-cell activation; it is essential for the activation of the IL-2 gene (6–9). The GM-CSF and IL-3 genes, however, appear to be subject to a more complex mode of regulation than IL-2. We observed that the proximal promoters of these genes responded poorly to signals normally delivered via the T-cell receptor and were relatively insensitive to CsA (see below). This suggests that a distal regulatory element(s) may participate in the activation of the GM-CSF/IL-3 locus and be a target for CsA inhibition.

Transcriptional regulatory elements frequently adopt altered chromatin structures, termed DNase I hypersensitive (DH) sites, which can be detected within nuclei on the basis of their enhanced accessibility to DNase I (10). DH sites most likely represent nucleosome-free regions where specific DNA binding proteins associate with DNA and exclude nucleosomes. In this study, we mapped DH sites across the human GM-CSF/IL-3 locus and located an inducible DH site† in the intergenic region, which functioned as a powerful CsA-inhibitable enhancer.

MATERIALS AND METHODS

Plasmid Construction. pHGM0.6 was created by inserting the multiple cloning site AGCTGATATCTCGAGATC-TGGGCCCGGATCCTGCAGGCCTAAGCTTCGCGAG-GTCACC into the *Hin*dIII site at the 5′ end of the 0.6-kb fragment of the human GM-CSF promoter in the chloramphenicol acetyltransferase (CAT) reporter gene plasmid pC-SFp1+ (11). Subsequent derivatives of pHGM0.6 incorporating additional fragments of the human GM-CSF/IL-3 locus contained 2.7-kb *Bgl* II/*Hin*dIII (pHGM3.3), 2.5-kb *Bam*HI/*Hin*dIII (pHGM3.1), 2.0-kb *Bgl* II/*Hin*dIII (pHGM2.6), or 0.7-kb *Bgl* II (pHGMB716) fragments of λJ1-16 DNA (2) inserted into the corresponding sites upstream of the promoter in pHGM0.6. pHGMN3 contained three head-to-tail copies of the GM550 element GATCTCTTATTATGACTCTTGCTT-TCCTCCTTTCA in the *Bgl* II site of pHGM0.6. pHIL3 contained a 5.2-kb *Hin*dIII/*Ban* II fragment of λ-66 DNA (a gift from S. Clark and Y.-C. Yang) from upstream of the human IL-3 gene inserted into the *Hin*dIII/*Sal* I sites of pBLCAT3 (12). pHIL3B716 had the 716-bp *Bgl* II fragment inserted into the *Hin*dIII site of pHIL3. All DNA fragments were inserted in the same orientation relative to the promoters as they exist in the GM-CSF/IL-3 locus.

DH Site Analyses. Nuclei isolations and DNA digestions were performed essentially as described (5). Briefly, nuclei were isolated from Jurkat cells by lysis in 0.1% Nonidet P-40 and resuspended to ≈0.4 mg/ml (optical density at 260 nm of an aliquot in 1 M NaOH = 10 absorbance units/ml). Aliquots of nuclei were digested for 3 min at 22°C with DNase I (Worthington) at 2–20 units/ml in nuclei isolation buffer containing 1 mM $CaCl_2$. DNA was purified, digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, blotted onto a Hybond N membrane (Amersham), and hybridized to the ^{32}P-labeled 1.4-kb *Bam*HI fragment of λJ1-16 DNA indicated

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Abbreviations: GM-CSF, granulocyte–macrophage colony-stimulating factor; IL-2 and IL-3, interleukins 2 and 3; CsA, cyclosporin A; DH, DNase I hypersensitive; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate.

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†The sequence reported in this paper has been submitted in the GenBank data base (accession no. L07488).

in Fig. 1. One representative sample was selected from each DNase I titration. The extent of DNase I cutting at the DH site was determined by quantitation of ^{32}P emissions with a Molecular Dynamics PhosphorImager.

Analyses of mRNA Expression. GM-CSF and IL-3 mRNA accumulation, relative to β-actin, was determined by RNase protection assay (13). SP6 RNA polymerase transcription templates for the preparation of ^{32}P-labeled RNA probes used in RNase protection assays contained the terminal 207 bp of the human GM-CSF gene (gift from G. Goodall), the 301-bp *Sma* I/*Pst* I fragment of the human IL-3 gene, and the 130-bp *Pst* I/*Sma* I fragment of the human β-actin gene. mRNA levels were quantitated with a Molecular Dynamics PhosphorImager. The levels of IL-3 and GM-CSF mRNA were normalized as the ratio over the level of β-actin detected in each assay and displayed as a percentage of the maximum level induced in each series of experiments.

Cell Transfections. Jurkat cells (5×10^6) were transfected by electroporation with 10 μg of DNA and cultured for 16–24 h before stimulating with phorbol 12-myristate 13-acetate (PMA) at 20 ng/ml plus 2 μM A23187, in the presence or absence of 0.1 μM CsA. After 6 h the cells were washed to remove the stimulus and cultured for 16–24 h in fresh medium before harvesting cytoplasmic protein for CAT assay (14). Relative CAT activity and standard errors were calculated from at least four experiments by using unstimulated pHGM0.6 set at a value of 1 as a reference. At least two independently derived clones of each construct were tested. The fold induction in response to PMA and A23187 was calculated relative to each unstimulated construct.

Gel Electrophoretic Mobility Shift Assays. Mobility shift assays were performed as described (15) using 4% polyacrylamide gels in 25 mM Tris borate/0.5 mM EDTA. Nuclear extracts were prepared as described (16) from Jurkat cells with and without stimulation for 3 h with PMA at 20 ng/ml and 2 μM A23187. In each assay, 4 μg of nuclear protein, 5 μg of poly(dI-dC), and 0.2 ng of DNA probe were incubated for 25 min at 22°C in 18 μl of 30 mM NaCl, 30 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 20 mM Hepes (pH 7.9), 0.1 mM phenylmethylsulfonyl fluoride, aprotinin at 5 μg/ml, and leupeptin at 5 μg/ml. Assays in Fig. 5*b* were performed in the presence and absence of 25 ng of specific competitor DNA. GM170, GM330, GM400, and GM550 probes and competitors corresponded to the DNA segments indicated in Fig. 2*b*. The human IL-2 gene NFAT probe and competitor (gift of G. Crabtree) encompassed the sequence GGAGGAAAAACTGTTTCATACAGAAGGCGT. The human stromelysin gene AP1 probe and competitor encompassed the sequence GCAAGGATGAGTCAAGCTGCGGGTGATCC. The nonspecific DNA competitor had the sequence TCGCCAATGAGCTCCCGGGTCGACTGCAGAAGCTTC.

RESULTS

An Inducible DH Site Lies Upstream of the GM-CSF Gene. Elements required for the correct regulation of the GM-CSF/IL-3 locus were sought by mapping DH sites (10) in the Jurkat human T-cell line before and after stimulation with PMA and the Ca^{2+} ionophore A23187. These stimuli mobilize protein kinase C and increase free cytoplasmic Ca^{2+}, respectively, and thus mimic at least some aspects of T-cell receptor activation (1). We mapped DH sites across ≈40 kb of DNA extending from an *Eco*RI site 12 kb upstream of the IL-3 gene to a *Bgl* II site 12 kb downstream of the GM-CSF gene. Just one inducible DH site, located 2.8–3.0 kb upstream of the GM-CSF gene, was detected in Jurkat cells (Figs. 1*a* and 2*a*); this site was also inducible in peripheral blood T cells (not shown). In addition, at least seven constitutive DH sites were detected in the vicinity of the IL-3 gene (Fig. 2*a*; data not shown).

Maximal induction of the DH site upstream of the GM-CSF gene required two signals, provided here by PMA and the Ca^{2+} ionophore, as did the induction of GM-CSF and IL-3 gene mRNA accumulation (Fig. 1*a*). The DH site appeared 1–2 h before the onset of GM-CSF and IL-3 mRNA accumulation (Fig. 1*b*), and its induction was blocked by both the transcription inhibitor dichlorobenzimidazole riboside and the translation inhibitor cycloheximide (Fig. 1*a*). Significantly, the immunosuppressant CsA, which inhibited both GM-CSF and IL-3 transcription, also blocked induction of the DH site (Fig. 1*a*). The DH site required ongoing induction of nuclear factors for its continued presence, since a significant decrease in intensity occurred 4 h after either addition of CsA or removal of the stimulus (Fig. 1*c*).

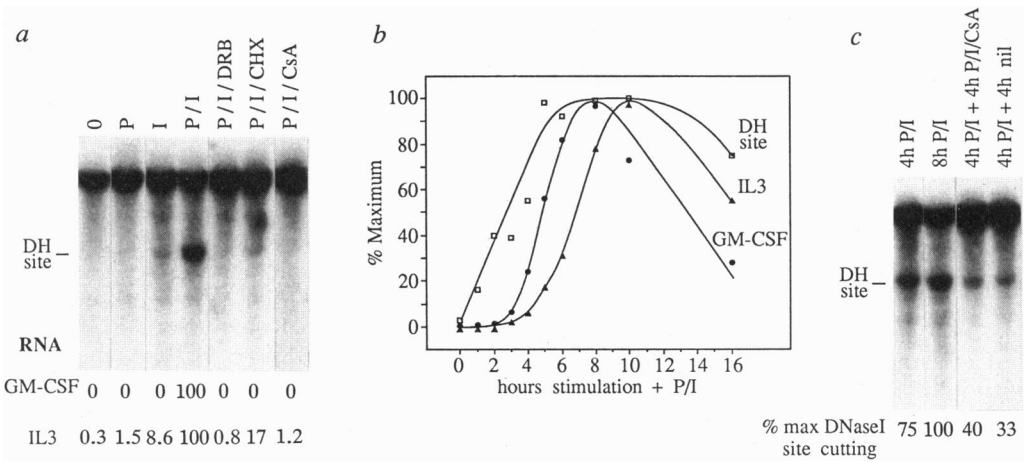

FIG. 1. An inducible DH site exists upstream of the GM-CSF gene. (*a*) Induction of the DH site and mRNA expression in Jurkat cells following a 4-h incubation with the indicated stimuli. The upper band is the intact 9.4-kb *Eco*RI fragment encompassing the GM-CSF gene, while the lower band is a 3.9-kb subfragment generated by DNase I digestion (see Fig. 2*a*). The numbers below indicate relative accumulation of GM-CSF and IL-3 mRNA in the same cultures. (*b*) Time course of induction of the DH site (□) and GM-CSF (●) and IL-3 (▲) mRNA accumulation after stimulation with PMA and Ca^{2+} ionophore. (*c*) The stability of the DH site following induction was tested by either adding CsA after 4 h of an 8-h PMA/Ca^{2+} ionophore stimulation period or by removing the stimulus at 4 h and incubating for an additional 4 h in medium alone (nil). The first lane provides a measure of the extent of DH site formation after just 4 h of stimulation. 0, unstimulated; P, PMA at 20 ng/ml; I, 2 μM Ca^{2+} ionophore A23187; DRB, 100 μM dichlorobenzimidazole riboside; CHX, cycloheximide at 20 μg/ml; CsA, 0.1 μM. Inhibitors were added 10 min before PMA and A23187.

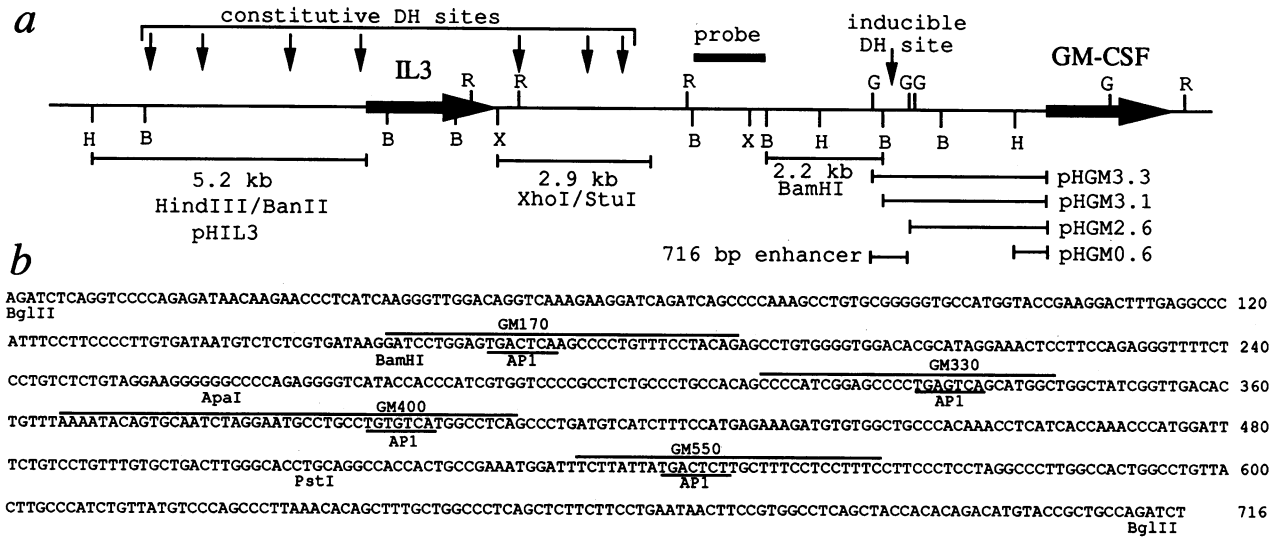

FIG. 2. Location and sequence of the inducible DH site. (*a*) Restriction enzyme map of the human GM-CSF/IL-3 locus displaying the location of DH sites (vertical arrows). The map was derived from the phage clones λJ1-16 (2) and λ-66 (obtained from Y.-C. Yang and S. Clark) and the plasmid pCH5.2 (11). The solid bar above the map represents the DNA probe used to map the inducible DH site. The bars below indicate DNA fragments tested in subsequent transcriptional analyses. H, *Hin*dIII; B, *Bam*HI; R, *Eco*RI; X, *Xho* I; G, *Bgl* II. (*b*) DNA sequence of the 716-bp *Bgl* II fragment spanning the DH site. AP1-like elements are underlined, whereas DNA probes and competitors used in subsequent electrophoretic mobility shift assays are indicated above the sequence. The DH site lies between the *Apa* I and *Pst* I sites. The DNA sequence was determined using an Applied Biosystems automated sequencer, by sequencing each strand of overlapping DNA clones derived from λJ1-16.

GM-CSF and IL-3 Are Regulated by an Inducible CsA-Sensitive Enhancer. The inducible DH site was tested for transcriptional regulatory activity in transient transfection assays in Jurkat cells. Segments of up to 3.3 kb of DNA, extending upstream from the GM-CSF gene transcription start site, were linked to the CAT reporter gene (pHGM0.6–pHGM3.3; Fig. 2*a*). DNA segments that include the DH site (pHGM3.3 and pHGM3.1) had little constitutive activity, but mediated an 80- to 95-fold induction of CAT in response to PMA plus Ca^{2+} ionophore (Fig. 3*a*). In contrast, very weak induction of CAT was supported by GM-CSF promoter segments lacking the DH site (pHGM2.6 and pHGM0.6; Fig. 3*a*). Furthermore, a 716-bp *Bgl* II fragment of DNA encompassing the DH site functioned as a powerful inducible enhancer when linked directly to the 0.6-kb GM-CSF proximal promoter fragment in both its normal (pHGMB716; Fig. 3*b*) and reverse orientation (not shown). No additional enhancers were detected upon testing a 2.2-kb *Bam*HI fragment immediately upstream of the inducible DH site or a 2.9-kb *Xho* I/*Stu* I fragment encompassing three constitutive DH sites just downstream of the IL-3 gene (Fig. 2*a*; data not shown).

The enhancer was also required for efficient induction of the IL-3 promoter in transient transfection assays. A CAT gene plasmid containing 5.2 kb of the IL-3 promoter and upstream DNA, including four upstream DH sites, had a moderate basal activity, but was only weakly induced by PMA and the Ca^{2+} ionophore (pHIL3; Figs. 2*a* and 3*b*). However, a reduced basal activity and a 10-fold induction of CAT was obtained when the 716-bp enhancer was coupled to the IL-3 promoter, 5.2 kb upstream of the CAT gene (pHIL3B716; Fig. 3*b*). Similar results were obtained when the enhancer was placed either upstream of the promoter or downstream of the CAT gene in an IL-3 promoter/CAT gene construct containing just the 315-bp *Stu* I/*Ban* II fragment from immediately upstream of the IL-3 transcription start site (data not shown).

The enhancer appeared to be the major target for CsA in the GM-CSF/IL-3 locus, since CsA suppressed the enhancer-driven activation of both promoters (pHGMB716 and pHIL3B716; Fig. 3*b*). In contrast, CsA had little inhibitory effect on the GM-CSF promoter alone (pHGM0.6; Fig. 3*b*) and unexpectedly led to a slightly increased induction of both the 5.2-kb (pHIL3; Fig. 3*b*) and 315-bp (data not shown) IL-3 promoter constructs used in this study.

The Enhancer Binds AP1 and NFAT. The 716-bp enhancer fragment was sequenced and found to encompass four consensus binding sequences for the inducible CsA-resistant transcription factor AP1 (17, 18) (Fig. 2*b*). Two of these sites (located at positions 170 and 330) match perfectly the TGAGTCA AP1 consensus, while the other two (located at positions 400 and 550) have a single base mismatch. The AP1 element at position 550 is, however, identical to a functional AP1 element in the IL-2 promoter (19, 20) (Fig. 4). Furthermore, this AP1 element is contained within a broader region (segment GM550; Fig. 2*b*) homologous to an NFAT site located in a more distal region of the IL-2 promoter (Fig. 4). NFAT could, therefore, also participate in the CsA-sensitive activation of the GM-CSF and IL-3 genes in Jurkat cells. Indeed, three linked copies of the GM550 segment functioned as a strong inducible enhancer inhibited by CsA, when placed upstream of the GM-CSF promoter (pHGMN3; Fig. 3*b*).

To identify the transcription factors that mediate enhancer function, we assayed fragments of the enhancer in electrophoretic mobility shift assays with Jurkat cell nuclear extracts prepared from unstimulated cells and cells stimulated in the presence and absence of CsA (Fig. 5*a*). Complexes induced by PMA and Ca^{2+} ionophore were formed with four DNA segments encompassing AP1 sites (GM170, GM330, GM400, and GM550 as in Fig. 2*b*). Each probe formed a CsA-resistant complex that comigrated with a stromelysin gene (21) AP1 complex. Furthermore, all four AP1 sites were protected by recombinant c-jun protein (18), a component of AP1, in DNase I footprinting assays (data not shown). Three of the probes (GM170, GM330, and GM550) also formed inducible CsA-sensitive complexes that comigrated with an inducible complex formed with the distal NFAT site from the human IL-2 gene (6, 8, 22). The IL-2 NFAT probe formed two inducible CsA-inhibitable complexes similar to those detected with a mouse IL-2 gene NFAT site (22). The lower of these complexes comigrated with AP1 and may be a previously described component of NFAT that lacks AP1 (22). All

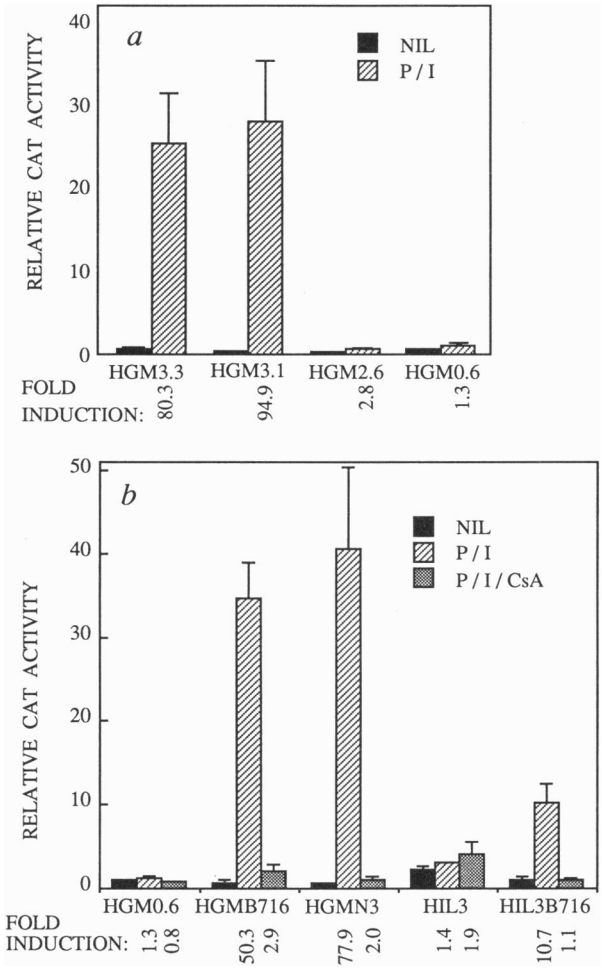

FIG. 3. Identification of a transcriptional enhancer encompassing the inducible DH site. (*a*) Stepwise deletions of a 3.3-kb segment of DNA upstream of the GM-CSF gene reveal an inducible enhancer located between 3.1 and 2.6 kb upstream. CAT gene constructs contained 3.3, 3.1, 2.6, or 0.6 kb of DNA from upstream of the GM-CSF transcription initiation site. (*b*) The 716-bp *Bgl* II fragment located 2.6–3.3 kb upstream of the GM-CSF gene mediates inducible CsA sensitive activation of the GM-CSF (pHGMB716) and IL-3 (pHIL3B716) promoters. pHIL3 contains 5.2 kb of DNA from immediately upstream of the IL-3 gene transcription start site. pHGMN3 contains three copies of GM550 (Fig. 2*a*) inserted upstream of the GM-CSF promoter in pHGM0.6. P, PMA; I, A23187; NIL, no additions.

six probes also bound constitutively expressed factors, which were not investigated further as they may be nonspecific.

The nature of the inducible complexes was further examined by using the stromelysin gene AP1 site and the IL-2 gene NFAT site as specific competitors (Fig. 5*b*). The AP1 competitor largely or completely inhibited binding of AP1-like factors to all four AP1 sites in the enhancer. The formation of the upper NFAT-like complexes was, in each case, inhibited not only by the NFAT competitor but also by the AP1 competitor. Conversely, the GM170, GM330, and GM550 NFAT-like elements efficiently inhibited the binding of both

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IL2 AP1 site                  -144 AAGAGTCAT -136
                                   *********
GM550 NFAT/AP1  566 GAAAGGAGGAAAGCAAGAGTCATAATA  540
                    ************    *  *****
IL2 NFAT site  -282 GAAAGGAGGAAAAACTGTTTCATACAG -256
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FIG. 4. Alignment of the GM550 NFAT/AP1-like element located at positions 566–540 in the enhancer with AP1 and NFAT binding-sites in the human IL-2 promoter (5). Stars indicate regions of identity.

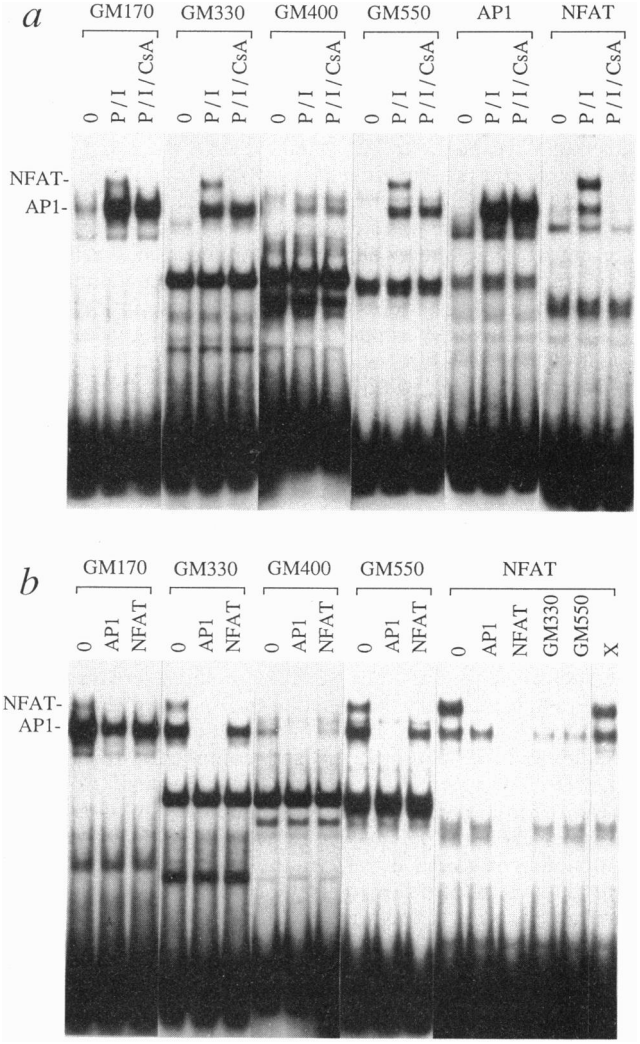

FIG. 5. Electrophoretic mobility shift assays of AP1 and NFAT-like elements in the enhancer. (*a*) Demonstration of inducible CsA-resistant AP1-like binding to the GM170, GM330, GM400, and GM550 elements and inducible CsA-sensitive NFAT-like binding to the GM170, GM330, and GM550 elements of the enhancer. The two right-hand panels served as controls and used the human stromelysin gene AP1 element (21) and the human IL-2 gene distal NFAT element (6–9). Each probe was assayed with nuclear extracts prepared from Jurkat cells that were either unstimulated (0), stimulated with PMA and Ca^{2+} ionophore (P/I), or stimulated with PMA and Ca^{2+} ionophore in the presence of 0.1 μM CsA (P/I/CsA). (*b*) Specific inhibition of AP1 and NFAT complexes in stimulated cell nuclear extracts by unlabeled competitor DNA. The probes are listed above, and the competitors are identified below each probe. X represents a nonspecific competitor.

of the NFAT-like complexes to the IL-2 gene NFAT site, whereas an unrelated DNA competitor (X) did not inhibit formation of any of the AP1 or NFAT-like complexes (Fig. 5*b*; data not shown).

DISCUSSION

Functional analyses of potential regulatory elements in the GM-CSF/IL-3 locus revealed a single enhancer that may normally be required for the coinduction of these genes in T cells. In Jurkat cells, the enhancer appeared to be the only element within this locus that responded significantly to stimulation by PMA and Ca^{2+} ionophore. Induction of the DH site within the enhancer preceded the onset of IL-3 and GM-CSF mRNA accumulation, suggesting that activation of

the IL-3 and GM-CSF proximal promoters requires prior activation of the enhancer.

Activation of the enhancer appeared to involve remodeling of its chromatin structure and binding of specific transcription factors. Since DH site induction and enhancer activation occurred in response to PMA and A23187, the enhancer is a likely target for protein kinase C and Ca^{2+}-transduced signals resulting from activation of the T-cell receptor. Consistent with this view is the observation that AP1 and NFAT, which interact with the enhancer, are both induced in T cells after T-cell receptor stimulation. Interestingly, the enhancer may also function in the repression of basal transcription.

DNA binding and competition studies strongly suggested that four sites within the enhancer associate, to differing degrees of affinity, with the AP1 family of transcription factors. Two of these sites (GM170 and GM330) perfectly match the AP1 consensus and formed AP1-like complexes almost as efficiently as the stromelysin gene AP1 element used as a reference in this study. The GM550 AP1 element, which is identical to the IL-2 gene AP1 site, bound AP1 less efficiently, whereas the GM400 AP1 element bound AP1 only very weakly. Further studies are required to determine whether all of these AP1 sites are functional components of the enhancer.

Good evidence was obtained suggesting that the T cell-specific transcription factor NFAT associates with three sites in the enhancer. Each site satisfied the operational definition of NFAT sites, in that they formed inducible CsA-sensitive complexes resembling NFAT and competed with an IL-2 gene NFAT site for NFAT binding. At least one of these sites (GM550) functioned as a powerful inducible, CsA-inhibitable enhancer element (pHGMN3; Fig. 3*b*).

Induction of NFAT occurs via the assembly of two distinct components, in the following fashion. A preexisting cytosolic component (NFATc) migrates in a CsA-inhibitable fashion to the nucleus, where it associates with a newly synthesized component (NFATn) that is not inhibited by CsA (9). While the identity of NFATc is unknown, Jain *et al.* (22) have proposed that NFATn is an AP1-like factor. Accordingly, we and others (22) have observed that AP1 DNA competitors inhibit both NFAT and AP1 binding. Interestingly, all three NFAT-like elements defined here encompass AP1 sites. From this we not only conclude that an AP1-like factor is an essential component of the NFAT complex but further predict that the AP1-like component of NFAT interacts directly with AP1-like consensus elements in at least some NFAT sites. Note that the distal IL-2 NFAT site also includes an AP1-like element (TGttTCA; Fig. 4), which appears unable to bind AP1 independently, but may represent the site of interaction with an AP1-like component of the NFAT complex.

NFAT binding sites also typically encompass homopurine segments, and an ets-like GGA core sequence (23) within this region may be essential (22). Significantly, the GM550 and IL-2 NFAT sites share identity across 12 bp of the homopurine segment. The GM330 NFAT site also retains the GGA motif in the same position relative to the AP1-like motif as it exists in the IL-2 and GM550 sites, whereas the GM170 NFAT site includes a conserved AGGAAA motif at a slightly greater distance. NFATc is a good candidate for the protein that interacts with the purine region, although it is yet to be established whether NFATc is itself a DNA binding protein. We and others have, however, observed formation of a second faster migrating complex with IL-2 NFAT probes (Fig. 5; ref. 22), which is CsA-sensitive and does not appear to contain AP1 and could therefore represent the NFATc component.

Unlike the IL-2 NFAT site, the GM170, GM330, and GM550 NFAT sites each had the capacity to bind AP1 independently of NFAT. These sites may represent an additional class of NFAT sites that retain some function in cells other than T cells, which lack the T cell-specific protein NFATc. It will be of interest to determine whether the enhancer mediates activation of the GM-CSF gene in the wide range of cell types that express GM-CSF but lack NFAT.

The enhancer can also account for the CsA sensitivity of the locus, via mechanisms similar to those previously observed for the IL-2 gene (5–9), which involve inhibition of induction of NFAT and suppression of a DH site. The enhancer remains the only element identified in the locus that is both highly inducible and inhibitable by CsA. By inhibiting the enhancer, and consequently GM-CSF and IL-3 expression, CsA may be suppressing elements of the immune response that contribute to graft rejection, autoimmunities, and allergic diseases such as asthma (24).

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