MOLECULAR AND CELLULAR BIOLOGY, Oct. 1992, p. 4357–4363
0270-7306/92/104357-07$02.00/0

Vol. 12, No. 10

Regulation of T-Cell Lymphokine Gene Transcription by the Accessory Molecule CD28

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Received 22 May 1992/Returned for modification 11 June 1992/Accepted 7 July 1992

T-cell activation results in the production of multiple lymphokines. Efficient lymphokine gene expression appears to require both T-cell antigen receptor (TCR) signal transduction and an uncharacterized second or costimulatory signal. CD28 is a T-cell differentiation antigen that can generate intracellular signals that synergize with those of the TCR to increase T-cell activation and interleukin-2 (IL-2) gene expression. In these studies, we have examined the effect of CD28 signal transduction on granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL-3), and gamma interferon (IFN-γ) promoter activity. Stimulation of CD28 in the presence of TCR-like signals increases the activity of the GM-CSF, IL-3, and IFN-γ promoters by three- to sixfold. As previously demonstrated for the IL-2 promoter, the IL-3 and GM-CSF promoters contain distinct elements of similar sequence which specifically bind a CD28-induced nuclear complex. Mutation of the CD28 response elements in the IL-3 and GM-CSF promoters abrogates the CD28-induced activity without affecting phorbol ester- and calcium ionophore-induced activity. UV cross-linking indicates that the CD28-induced nuclear complex contains polypeptides of approximately 35, 36, and 44 kDa. These studies indicate that the TCR and CD28-regulated signal transduction pathways coordinately regulate the transcription of several lymphokines and that the influence of CD28 signals on transcription is mediated by a common complex.

T lymphocytes are activated by a complex interaction with antigen-presenting cells to perform effector functions, including lymphokine secretion and cytolysis. Interaction of the T-cell receptor (TCR) with antigen in the context of major histocompatibility complex molecules on the surface of the antigen-presenting cells leads to activation of tyrosine kinase and phospholipase C activities which contribute to cellular responses (16, 39). However, in addition to the TCR, other T-cell surface receptors appear to be required for T-cell activation. Some cell surface molecules are required to increase the adhesion between the T cell and the antigen-presenting cell (35). Other cell surface receptors deliver an uncharacterized second or costimulatory signal (30). One candidate molecule for the receptor which generates this costimulatory signal is CD28. CD28 is a 44-kDa glycoprotein member of the immunoglobulin superfamily that is expressed as a homodimer on the surface of most T cells (1, 13). Treatment of T cells with monoclonal antibodies (MAbs) directed against CD28 induces an unidentified but distinct signal transduction pathway that synergizes with TCR-generated signals to increase both T-cell proliferation and lymphokine production (19, 40). CD28 signal transduction increases the mRNA level and secretion rate for a variety of lymphokines, including interleukin 2 (IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, tumor necrosis factor α, gamma interferon (IFN-γ), and lymphotoxin (15, 37). Whereas CD28 has been reported to regulate the expression of lymphokines by increasing their mRNA stability (20), we and others have found that CD28 signal transduction markedly increases IL-2 promoter activity (8, 38).

CD28 stimulation induces the binding of a nuclear protein complex to an element of the IL-2 promoter located between −160 and −152 relative to the transcription start site which is required for the CD28-induced promoter activity (8). Multiple T-cell lymphokines have been shown to be regulated by CD28 signal transduction, and several lymphokine promoters contain sequences similar to that of the IL-2 CD28 response element (CD28RE). In this study, we have examined whether the GM-CSF, IL-3, and IFN-γ promoters are also responsive to CD28 stimulation. Our results indicate that CD28 signal transduction does increase the activity of the GM-CSF, IL-3, and IFN-γ promoters by three- to sixfold and that the IL-2 CD28RE (−160 to −152)-homologous sequences contained within the promoters are required for CD28-induced activity.

MATERIALS AND METHODS

Oligonucleotides and plasmids. All oligonucleotides were synthesized on an Applied Biosystems model 394 DNA synthesizer. Full-length oligonucleotides were purified by polyacrylamide gel electrophoresis. Oligonucleotides were kinase treated and annealed as described previously (23). The 5′ flanking region and promoter of human IL-3 was generated from genomic DNA by using a polymerase chain reaction (PCR) (28) based on previously described constructs (34). The human GM-CSF promoter constructs were generated by using PCR of −626 GM-CSF-CAT, a gift of J. Gasson and S. D. Nimer, University of California, Los Angeles (5). The IL-3 and GM-CSF promoter fragments were ligated into the luciferase plasmid pXP2 (27), using the *Bam*HI and *Xho*I sites. Mutation of the CD28RE sites of IL-3 (−324 to +41)-Luc, GM-CSF (−374 to +34)-Luc, and GM-CSF (−274 to +34)-Luc was performed by using PCR as described previously (14). IFN-γ (−214 to +42)-CAT was the generous gift of H. Fox and T. Parslow, University of California, San Francisco.

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Jurkat was maintained in RPMI 1640 supplemented with 10% fetal calf serum as previously described (8). Jurkat cells were transiently transfected by using DEAE-dextran (8). After 20 h, the cells were stimulated as indicated for 8 h, and cytoplasmic extracts were prepared. The determination of luciferase and chloramphenicol acetyltransferase (CAT) was performed as described previously (8).

Preparation of nuclear extracts. Nuclear extracts were prepared by a modification of the method of Dignam et al. (7). Nuclear proteins were extracted with 350 mM NaCl–20% glycerol–0.2 mM EDTA–1.5 mM $MgCl_2$–1 mM dithiothreitol–1 μM phenylmethylsulfonyl fluoride–20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.9) for 40 min at 4°C. The extract was dialyzed against 20% glycerol–100 mM NaCl–1 mM dithiothreitol–1 μM phenylmethylsulfonyl fluoride–0.1 mM EGTA–10 mM HEPES (pH 7.9) for 6 h. Protein concentrations were determined by the Bradford method (3).

Mobility shift electrophoretic assay. Duplex oligonucleotides corresponding to various regions of the lymphokine promoters were annealed in 60 mM NaCl–20 mM Tris hydrochloride (pH 7.6). The oligonucleotides were end labeled with [γ-^{32}P]ATP, using T4 polynucleotide kinase. For binding reactions, 0.2 to 0.5 ng of radiolabeled double-stranded oligonucleotide (5,000 to 20,000 cpm) was mixed with 1 μg of nuclear extract in 150 mM NaCl–20 mM KCl–30 mM Tris (pH 7.6)–15% glycerol–50 μg of bovine serum albumin per ml (final volume, 10 μl); 2 μg of poly(dI-dC) was added as a nonspecific competitor. For binding competition experiments, competitor oligonucleotides were added prior to addition of the radiolabeled probe. Reactions were incubated for 20 min at 20°C. The reactions were analyzed on 6% polyacrylamide gels (22.5 Tris-borate [pH 8.0], 1 mM EDTA) run at 100 V.

UV cross-linking of nuclear proteins. For UV cross-linking, 20 μg of nuclear extract was mixed with 5 ng of [^{32}P]ATP-labeled oligonucleotide probe (2×10^6 cpm) and subjected to a mobility shift assay. After electrophoresis for 2 h at 100 V, the gel was UV irradiated for 20 min (305 nm). The gel was exposed to film, and the CD28-induced nuclear complex (CD28RC)-specific bands were then excised and analyzed on sodium dodecyl sulfate (SDS)–10% polyacrylamide gels.

RESULTS

The CD28-induced increase in IL-2 promoter activity has previously been mapped to an element located between −161 and −151 relative to the transcription start site (8). The promoter regions of several other T-cell lymphokines contain very similar conserved sequences (Table 1). To determine whether these promoters were also responsive to CD28 stimulation, we transiently transfected the T-cell line Jurkat with constructs containing the promoters and a portion of the 5' flanking regions of the GM-CSF, IL-2, IL-3, and IFN-γ genes directing expression of the reporter genes for luciferase or CAT. In agreement with earlier studies, basal activity of these lymphokine promoters was low, and treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin increased reporter gene activity by 7- to 35-fold (5, 25, 26, 34). As previously observed, addition of an anti-CD28 MAb increased IL-2 promoter activity in PMA- and ionomycin-treated cells by 5.2 ± 0.4-fold (8, 38) (Fig. 1A). CD28 stimulation also increased reporter gene activity in cells transfected with the GM-CSF, IL-3, and IFN-γ promoter constructs. Reporter gene activity was increased 5.4 ± 0.7-fold for GM-CSF (−374 to +34)-Luc-transfected cells (Fig. 1B), 4.1 ± 0.3-fold for IL-3 (−322 to +41)-Luc transfected cells (Fig. 1C), and 3.1 ± 0.4-fold for IFN-γ (−214 to +42)-CAT-transfected cells (Fig. 1D). The increased promoter activity appeared to be a specific response to CD28 stimulation, since treatment with an anti-class 1 histocompatibility antigen (HLA) MAb had only modest effects on the reporter gene activity. Since the IFN-γ (−214 to +42)-CAT construct was only weakly induced in Jurkat cells, we decided to focus our studies on the GM-CSF and IL-3 promoters.

TABLE 1. Conserved sequences in the 5' flanking regions of lymphokine genes

Gene[a]	Region[b]	Sequence[c]
hIL-2	−160 to −152	GAAATTCCA
mIL-2	−162 to −154	GAAATTCCA
hGM-CSF	−94 to −86	GAgATTCCA
mGM-CSF	−106 to −98	GAgATTCCA
hIL-3	−117 to −109	GAggTTCCA
mIL-3	−115 to −105	GAggTTCCA
hG-CSF	−188 to −178	GAgATTCCA
mG-CSF	−192 to −181	GAgATTCCc
hγ-IFN	−161 to −153	GAAAcTCtA
mγ-IFN	−168 to −160	GAAAcTCtA

[a] The prefixes h and m indicate human and murine, respectively.
[b] Numbering is relative to the transcription start site.
[c] Sequences are compared with the human IL-2 gene sequence. Nonconserved bases are shown in lowercase letters.

To address whether the sequences similar to the IL-2 CD28RE listed in Table 1 actually were capable of binding the CD28RC, we performed gel shift analysis using oligonucleotide probes that corresponded to −124 to −100 of the IL-3 promoter and −103 to −79 of the GM-CSF promoter (Fig. 2). Nuclear extract from untreated Jurkat cells formed several complexes with the IL-3 (−124 to −100) oligonucleotide probe (Fig. 2A). Treatment with PMA and ionomycin did not appreciably change the binding pattern (Fig. 2A, lanes 2 and 3). However, nuclear extract from cells treated with anti-CD28, PMA, and ionomycin formed an additional complex (Fig. 2A, lane 4). Competition with a 25- or 5-fold molar excess of unlabeled IL-3 (−124 to −100) oligonucleotide specifically inhibited binding of the CD28RC with the radiolabeled probe and partially inhibited one additional complex of more rapid mobility. Double-stranded oligonucleotides corresponding to IL-2 (−164 to −140) and GM-CSF (−103 to −79) also inhibited the CD28RC without affecting the intensity of any of the other bands. In contrast, a 100-fold molar excess of a double-stranded oligonucleotide corresponding to the IL-2 NF-AT site (−285 to −254) failed to affect binding of the CD28RC.

CD28 stimulation also induced binding to an oligonucleotide probe corresponding to GM-CSF (−103 to −79) (Fig. 2B, lane 4). This complex could also be specifically inhibited from binding by competition with a 25-fold molar excess of unlabeled GM-CSF (−103 to −79), IL-3 (−124 to −100), or IL-2 (−164 to −140) oligonucleotide (Fig. 2B, lanes 5 to 10). A 100-fold excess of an oligonucleotide corresponding to the IL-2 NF-AT element (−285 to −254) again failed to affect binding of the CD28RC to the GM-CSF (−103 to −79) oligonucleotide (Fig. 2B, lane 11). Moreover, the CD28RC that bound to the GM-CSF (−103 to −79) oligonucleotide was identical in mobility to that formed with the IL-3 (−124 to −100) oligonucleotide (Fig. 2B, lane 12) and with the complex formed with the IL-2 CD28RE (10). Therefore,

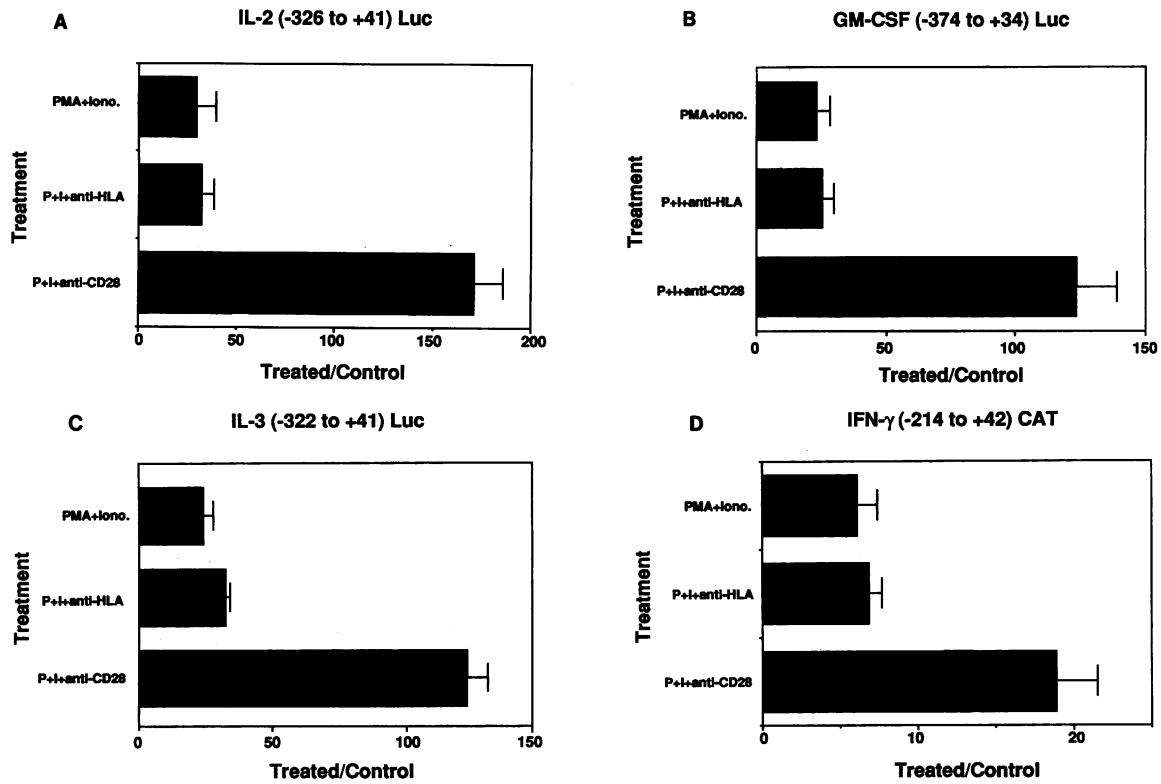

FIG. 1. Evidence that anti-CD28 induces increased T-cell lymphokine promoter activities in Jurkat cells treated with PMA and ionomycin. The induced activities of the indicated plasmid constructs were assayed in transiently transfected Jurkat cells. At 20 h after transfection, the cells were treated with the indicated combinations of PMA (P; 50 ng/ml), ionomycin (Iono or I; 1 μM), MAb W6/32 (anti-HLA; 50 ng/ml), or MAb 9.3 (anti-CD28; 50 ng/ml) for 8 h. Results are expressed as the mean fold induction ± standard error of treated cells over that of untreated cells. The results reflect the cumulative data from three independent experiments.

CD28 stimulation induced nuclear factors capable of binding similar sequences of the GM-CSF, IL-3, and IL-2 promoters that, by competition analysis and apparent mobility, appear to be identical.

To determine whether the sequences of the IL-3 and GM-CSF promoters that bound the CD28RC were required for the CD28-induced promoter activity, we performed site-directed mutagenesis of the CD28RC binding sites. It had previously been shown for the IL-2 CD28RE that mutagenesis of the CD28RE from GAAATTCC to GAAgcctC resulted in both the loss of binding by the CD28-induced nuclear complex and a 80 to 90% decrease of the CD28-induced IL-2 promoter activity (8). This mutation was introduced at −105 to −107 of the IL-3 (−324 to +41)-Luc plasmid, and Jurkat cells were transfected with the resulting mIL-3 (−324 to +41)-Luc construct. Both basal expression and PMA-plus-ionomycin-induced luciferase activity (Fig. 3, white bars) were similar or identical to those observed in cells transfected with the wild-type construct, but anti-CD28 stimulation induced only 15% of the increased luciferase activity observed with the wild-type construct (Fig. 3, black bars). This finding suggests that the CD28-augmented IL-3 promoter activity results from binding of the CD28RC to the −117 to −109 element.

However, mutation of −90 to −88 of GM-CSF (−374 to +34)-Luc resulted in only a 37% decrease in CD28-induced promoter activity (Fig. 4B, black bars). As this mutation in the GM-CSF (−103 to −79) oligonucleotide resulted in no detectable gel shift binding by the CD28-induced nuclear factors (10), these results suggested the presence of another CD28RE within the GM-CSF (−374 to +34) promoter region. Located between −300 and −292 of the GM-CSF promoter is the sequence GCCATTCCA, which is similar to the previously identified CD28RE sequences (Table 1; Fig. 4A). To determine whether this region was responsible for the remaining CD28-induced activity in the construct mGM-CSF (−374 to +34)-Luc, we deleted this region of the promoter. The resulting construct, GM-CSF (−274 to +34)-Luc, was transiently transfected into Jurkat cells. Treatment with PMA and ionomycin induced a 33 ± 4.9-fold increase in luciferase activity compared with unstimulated cells, and the addition of anti-CD28 caused the promoter activity to increase by a further 4.8 ± 0.6-fold (Fig. 4B). When the −90 to −88 site of GM-CSF (−274 to +34)-Luc was mutated (Fig. 4A), the resultant construct mGM-CSF (−274 to +34)-Luc had basal activity essentially identical to that of the wild-type construct and responded to PMA and ionomycin treatment as well as did the wild-type GM-CSF (−274 to +34)-Luc, but the CD28-induced increase was now only 17% of that of the wild-type sequence (Fig. 4B, black bars). These results indicate that both the −300 to −292 and −94 to −86 sequences of the GM-CSF promoter can act as CD28REs. Gel mobility shift experiments have also demonstrated that the GM-CSF −300 to −292 sequence can bind the CD28-induced nuclear complex but with a lower apparent affinity than does the −94 to −86 sequence (10).

In previous studies using the IL-2 promoter, the CD28-induced promoter activity was first detectable after 2 to 4 h

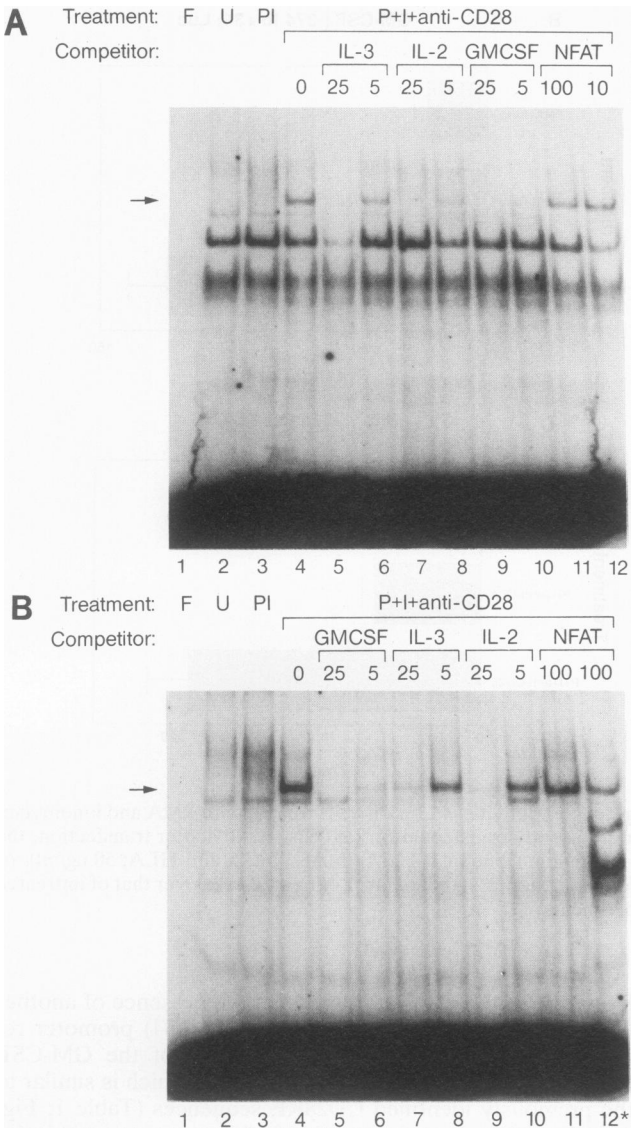

FIG. 2. Binding of the CD28RC to distinct elements of the IL-3 and GM-CSF promoters. A radiolabeled double-stranded oligonucleotide (0.2 ng per lane) corresponding to either IL-3 (−124 to −100) (A) or GM-CSF (−103 to −79) (B) was used in DNA-protein binding assays. F, probe alone; U, nuclear extract from untreated Jurkat cells; PI, extract from cells treated for 2 h with 1 μM ionomycin and 50 ng of PMA per ml; P+I+anti-CD28, extract from cells treated for 2 h with 1 μM ionomycin, 50 ng of PMA per ml, and 50 ng of MAb 9.3 (anti-CD28) per ml. Binding reaction mixtures contained either no competitor (lanes 1 to 4) or the indicated (5- to 100-fold) molar excess of unlabeled double-stranded oligonucleotide corresponding to IL-3 (−124 to −100 of the IL-3 promoter), IL-2 (−164 to −140 of the IL-2 promoter), GM-CSF (−103 to −79 of the GM-CSF promoter), or NF-AT (−285 to −254 of the IL-2 promoter). For lane 12 of panel B, a radiolabeled IL-3 (−124 to −100) probe was used to demonstrate that the CD28-induced complex that binds to the IL-3 probe is identical in mobility to that formed with the GM-CSF (−103 to −79) probe after gel shift analysis. The arrows indicate the CD28RCs.

(8). To determine the time course of CD28RC induction, we tested nuclear extracts for binding activity after treatment with PMA, ionomycin, and anti-CD28. CD28RC binding to the GM-CSF (−103 to −79) oligonucleotide was faintly

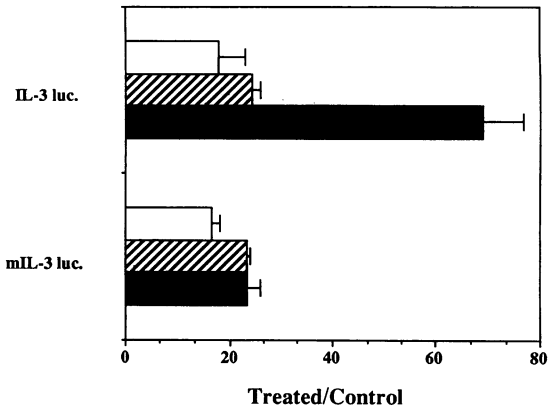

FIG. 3. Evidence that mutation of bases −107 to −105 renders the IL-3 promoter unresponsive to anti-CD28 treatment. Site-directed mutagenesis was used to mutate −117 to −109 of the IL-3 (−322 to +41)-Luc construct from GAGGTTCCA to GAGGcctCA. The resulting construct, mIL-3-Luc, and the wild-type IL-3-Luc were transiently transfected into Jurkat cells. After 20 h, the cells were treated with either 1 μM ionomycin and 50 ng of PMA per ml (white bars), ionomycin, PMA, and 50 ng of MAb W6/32 (anti-HLA) per ml (striped bars), or ionomycin, PMA, and 50 ng of MAb 9.3 (anti-CD28) per ml (black bars). After 8 h of treatment, the cells were harvested and the luciferase activity was determined. The data reflect results of three independent experiments. Error bars indicate standard error of the mean.

detectable after 1 h of treatment, became strongly induced after 2 h, and remained high after 4 h (Fig. 5).

To identify the molecular mass of the protein(s) that contacts DNA in the CD28RC-DNA complex, we performed photoaffinity cross-linking analysis. Utilizing the GM-CSF (−103 to −79) probe and a scaled-up binding reaction, the CD28RC was separated from free DNA by electrophoresis through a native polyacrylamide gel. The gel was irradiated in situ with UV light, and the CD28RC was analyzed on an SDS–10% polyacrylamide gel. Three major bands with apparent molecular masses of 35, 36, and 44 kDa were covalently linked to the GM-CSF probe (Fig. 6). In addition, there was a faint band of 66 kDa that was detectable after longer exposure to UV irradiation and may represent covalent linkage of both the 35- and 36-kDa factors to the DNA. Identical patterns of UV cross-linked proteins were found with use of the IL-3 (−124 to −100) and IL-2 (−164 to −140) oligonucleotide probes (10).

DISCUSSION

The regulation of lymphokine expression appears to involve several levels of control and signal input. Studies indicate that lymphokine production is regulated by both transcriptional and posttranscriptional mechanisms (33, 36). In T cells, signals derived from the TCR or agents such as PMA and ionomycin, which mimic the activation of phospholipase C by TCR signal transduction, constitute the primary regulatory pathway. However, several studies indicate that signal transduction from other cell surface receptors is important or perhaps required for T-cell lymphokine expression (30). CD28-generated signal transduction has previously been shown to synergize with TCR-like signals to increase the mRNA levels of several lymphokines (37). Initial studies reported that CD28 signal transduction increased lymphokine mRNA stability but could not detect

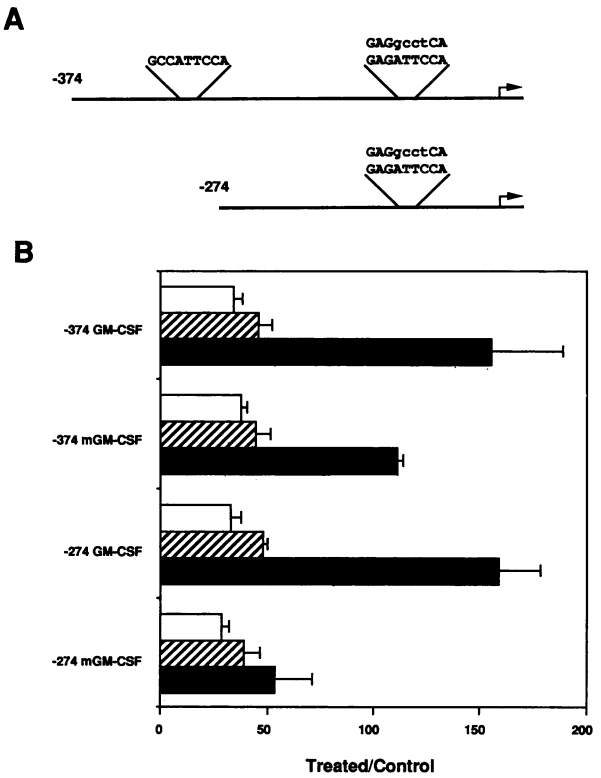

FIG. 4. Evidence that the GM-CSF promoter contains two CD28REs. (A) Schematic representation of GM-CSF promoter constructs and the putative CD28REs. Site-directed mutagenesis was used to mutate −91 to −88 of the GM-CSF (−374 to +34)-Luc and GM-CSF (−274 to +34)-Luc constructs from GAGATTCCA to GAGgcctCA. (B) Jurkat cells were transiently transfected with either GM-CSF (−374 to +34)-Luc (−374 GM-CSF), the mutant GM-CSF (−374 to +34)-Luc (−374 mGM-CSF), GM-CSF (−274 to +34)-Luc (−274 GM-CSF), or the mutant GM-CSF (−274 to +34)-Luc (−274 mGM-CSF). After 20 h, the cells were treated with either 1 μM ionomycin and 50 ng of PMA per ml (white bars), ionomycin, PMA, and 50 ng of MAb W6/32 (anti-HLA) per ml (striped bars), or ionomycin, PMA, and 50 ng of MAb 9.3 (anti-CD28) per ml (black bars). After 8 h of treatment, the cells were harvested and the luciferase activity was determined. The data reflect results of three independent experiments. Error bars indicate standard error of the mean.

any increase in the rate of transcription in nuclear run-on assays (20). However, nuclear run-on assays can be complicated by apparent constitutive signals due to repeat sequences in the lymphokine cDNA probes (4). Subsequent reports have shown that CD28-generated signals increase IL-2 promoter activity by about fivefold (8, 38). In this study, we have examined the role of CD28 signal transduction on the transcriptional activity of the GM-CSF, IL-3, and IFN-γ promoters. As was previously shown for the IL-2 promoter, CD28 signal transduction increases the promoter activity of these lymphokines by three- to sixfold relative to that maximally induced by the combination of PMA and ionomycin.

The CD28-induced increase in IL-2 promoter activity is dependent upon an element located between −160 and −152 relative to the transcription start site. Mutation of this site decreases the CD28-induced IL-2 promoter activity by 80 to 90% without affecting TCR- or PMA- and ionomycin-induced activity (8). Similar sequences have previously been

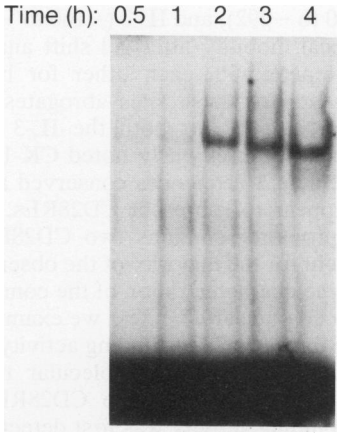

FIG. 5. Time course of CD28RC induction. A radiolabeled double-stranded oligonucleotide (0.2 ng per lane) corresponding to −103 to −79 of the GM-CSF promoter was incubated with nuclear extract from Jurkat cells treated for the indicated amount of time with 1 μM ionomycin, 50 ng of PMA per ml, and 50 ng of MAb 9.3 (anti-CD28) per ml. DNA-protein binding assays were performed by using a 6% native polyacrylamide gel.

identified as being conserved within the 5′ flanking region of several lymphokine promoters, including the GM-CSF and IL-3 promoters. These elements have been termed the conserved lymphokine element 1 or cytokine 1 (CK-1) element (24, 31). The role of the CK-1 elements in the regulation of T-cell lymphokine expression has been unclear since they do not appear to be involved in responses to TCR-like signals (25, 32). Recent studies indicate that in fibroblasts but not T cells, the granulocyte colony-stimulating factor CK-1 element activity is upregulated by treatment with tumor necrosis factor alpha α and IL-1β (18, 32). We have found that the CD28RC binds to both the IL-3 and GM-CSF CK-1 elements and to an additional 5′ site in the GM-CSF promoter (−300 to −292). The CD28RCs formed between the IL-3 (−117 to −109), GM-CSF (−94 to −86),

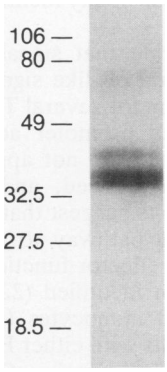

FIG. 6. UV cross-linking of the CD28RC-DNA complex. A ^{32}P-labeled GM-CSF oligonucleotide probe was incubated for 20 min with nuclear extract from Jurkat cells treated with ionomycin, PMA, and MAb 9.3 (anti-CD28) and then electrophoresed through a native polyacrylamide gel. The gel was irradiated in situ with UV light (305 nm) for 20 min, and the CD28RC-DNA complex was excised and analyzed on an SDS–10% polyacrylamide gel. The cross-linked polypeptides were visualized by autoradiography. The migration and apparent molecular masses in kilodaltons of protein standards are shown for reference.

GM-CSF (−300 to −292), and IL-2 (−160 to −152) elements all show identical mobility after gel shift analysis and can specifically compete with each other for binding to the CD28RC. Mutation of these sites abrogates the CD28-induced promoter activity for both the IL-3 and GM-CSF genes. Therefore, the previously noted CK-1 or conserved lymphokine element 1 sequences conserved among various lymphokines appear to represent CD28REs. Interestingly, the GM-CSF promoter contains two CD28REs, either of which is sufficient for the majority of the observed response.

To obtain some characterization of the components of the CD28RC, induced nuclear complex, we examined the kinetics of the CD28-induced DNA binding activity and used UV cross-linking to determine the molecular masses of the proteins that contact DNA at the CD28RE site. CD28-induced DNA binding activity was first detected after 1 h of treatment with anti-CD28, PMA, and ionomycin and became strongly induced after 2 h of treatment. Our subsequent experiments indicate that induction of binding activity requires protein synthesis (10). After UV cross-linking of ^{32}P-labeled CD28RE oligonucleotides to the CD28RC, three major bands with apparent molecular masses of 35, 36, and 44 kDa were found to be covalently linked to the DNA probes. At present, it is not clear whether any of these proteins correspond to known transcription factors.

It has previously been suggested that the CD28RC is NFκB-like since it will bind to an NFκB sequence (38). However, in light of our current results, it seems unlikely that the CD28RC consists of NFκB p50 and p65. First, treatment of Jurkat cells with PMA alone has previously been shown to strongly induce NFκB binding activity without any detectable binding to the IL-2 CD28RE (6, 8). While we have also observed that the CD28RC binds to an oligonucleotide corresponding to the immunoglobulin κ enhancer NFκB element, the mobilities of NFκB and the CD28-induced complex are significantly different after gel shift analysis (10). In this study, we found that the CD28RC binds with high affinity to the GM-CSF (−94 to −86) CK-1 site, while previous reports indicate that NFκB binds poorly if at all to this element (29, 32). Finally, the factors identified by our photoaffinity cross-linking have apparent molecular masses lower than those of any identified members of the *rel* gene family.

These results indicate that signal transduction through CD28 synergizes with TCR-like signals to greatly increase the rate of transcription for several T-cell lymphokines. The induction of increased promoter activity is mediated by specific CD28REs which do not appear to be involved in PMA- and ionomycin-induced, and hence TCR-induced, responses. These results suggest that CD28 signal transduction acts as a general pathway to upregulate lymphokine expression and T-cell effector function. A ligand for CD28, B7, has recently been identified (22). B7 is expressed on activated B cells and monocytes (11, 12). Incubation of anti-CD3-treated T cells with either B7 immunoglobulin Cγ1 fusion proteins or B7-expressing Chinese hamster ovary cells increases T-cell proliferation and IL-2 production (12, 21). Recent studies using either MAbs to block the interaction of B7 with CD28 or mutations within the IL-2 promoter CD28RE indicate that CD28 signal transduction is required for efficient T-cell cytotoxicity (2), alloactivation (17), and IL-2 production in response to staphylococcal enterotoxin stimulation (9). Taken together, these results suggest that the interaction of CD28 with B7 on antigen-presenting cells plays a critical role in regulating T-cell effector functions, including the transcriptional regulation of several lymphokines secreted by activated T cells.

ACKNOWLEDGMENTS

We thank Judith Gasson and Steven Nimer for the −626 GM-CSF CAT construct and Howard Fox and Tris Parslow for supplying the IFN-γ (−214 to +41)-CAT plasmid.

This work was supported in part by NIH grant GM-44493-01A2 to A.W.

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