Transcriptional Regulation of the Gene Encoding the Human C-type Lectin Leukocyte Receptor AIM/CD69 and Functional Characterization of Its Tumor Necrosis Factor-α-responsive Elements*

Manuel López-Cabrañes, Eduardo Muñoz, M. Valle Blázquez, Maria A. Ursa, Ana G. Santis, and Francisco Sánchez-Madrid†

From the Servicio de Inmunología and the ‡Unidad de Biología Molecular, Hospital de la Princesa, Universidad Autónoma de Madrid, 28006 Madrid, Spain and the §Departamento de Fisiología e Inmunología, Facultad de Medicina, Universidad de Córdoba, Avda Menéndez Pidal s/n, 14004 Córdoba, Spain

The human activation antigen CD69 is a member of the C-type animal lectin superfamily that functions as a signal-transmitting receptor. Although the expression of CD69 can be induced in vitro on cells of most hematopoietic lineages with a wide variety of stimuli, in vivo it is mainly expressed by T-lymphocytes located in the inflammatory infiltrates of several human diseases. To elucidate the mechanisms that regulate the constitutive and inducible expression of CD69 by leukocytes, we isolated the promoter region of the CD69 gene and carried out its functional characterization. Sequence analysis of the 5′-flanking region of the CD69 gene revealed the presence of a potential TATA element 30 base pairs upstream of the major transcription initiation site and several putative binding sequences for inducible transcription factors (NF-κB, Egr-1, AP-1), which might mediate the inducible expression of this gene. Transient expression of CD69 promoter-based reporter gene constructs in K562 cells indicated that the proximal promoter region spanning positions −78 to +16 contained the cis-acting sequences necessary for basal and phorbol 12-myristate 13-acetate-inducible transcription of the CD69 gene. Removal of the upstream sequences located between positions −78 and −38 resulted in decreased promoter strength and abolished the response to phorbol 12-myristate 13-acetate. We also found that tumor necrosis factor-α (TNF-α) is capable of inducing the surface expression of the CD69 molecule as well as the promoter activity of fusion plasmids that contain 5′-flanking sequences of the CD69 gene, suggesting that this cytokine may regulate in vivo the expression of CD69. In addition, cotransfection experiments demonstrated that the CD69 gene promoter can be activated by the NF-κB/Rel family members c-Rel and RelA. The deletion of the sequence spanning positions −255 to −170 abolished both the response to TNF-α and the transactivation by NF-κB. These results indicate that the NF-κB-binding site located at position −223 is necessary for the TNF-α-induced expression of the CD69 gene. Mobility shift assays showed that the two NF-κB motifs located in the proximal promoter region (positions −223 and −160) bind various NF-κB-related complexes, including the heterodimers p50/RelA and p50/c-Rel and homodimers of p50 (KBF-1) and RelA. Our findings help to explain the regulated synthesis of CD69 in vivo and suggest that TNF-α has a key role in the expression of this molecule at sites of chronic inflammation.

The human activation inducer molecule (AIM/CD69) is a phosphorylated disulfide-linked 27/33-kDa transmembrane homodimeric glycoprotein (Sánchez-Mateos and Sánchez-Madrid, 1991). The CD69 molecule is rapidly expressed on the surface of T-lymphocytes upon in vitro activation with a wide variety of agents, including anti-CD3/ T cell receptor and anti-CD2 mAbs, activators of protein kinase C, and phytohemagglutinin (Cebrián et al., 1988; Hara et al., 1986). Similarly, the expression of CD69 is inducible on the surface of NK cells, B-lymphocytes, and eosinophils (Hartnell et al., 1993; Lanier et al., 1988). In contrast, CD69 is constitutively expressed in vivo on platelets and on a small percentage of resident T- and B-cells of different lymphoid tissues (Sánchez-Mateos et al., 1989; Testi et al., 1990). Although a physiologic ligand for CD69 has not been identified so far, experiments with specific mAbs indicate that this antigen functions as a signal-transmitting receptor. Signals triggered by CD69 mAbs in T-lymphocytes induce increase in intracellular calcium concentration and result in the synthesis of different cytokines and their receptors, enhancement of the expression of c-myc and c-fos protooncogenes, and cell proliferation (Cebrián et al., 1988; Nakamura et al., 1989; Santis et al., 1992; Testi et al., 1989; Tugores et al., 1992). In NK cells and platelets, CD69 also acts as a triggering molecule, being involved in the redirected target cell lysis by interleukin-2-activated NK cells (Moretta et al., 1991) and in the induction of platelet aggregation, Ca2+ influx, and hydrolysis of arachidonic acid (Testi et al., 1990). The molecular cloning of cDNAs encoding human and mouse CD69 revealed that this antigen is a member of the Ca2+-dependent (C-type) animal lectin superfamily of type II transmembrane receptors (Hamann et al., 1993; López-Cabrera et al., 1993; Ziegler et al., 1993). This superfamily includes the human NKG2, rat and mouse NKR-P1, and mouse Ly-49 families of NK cell-specific antigens as well as the low avidity IgE...
The CD69 antigen is undetectable on peripheral blood lymphocytes; however, it is expressed at high levels by the majority of T-cells in the inflammatory cell infiltrates of several human diseases such as rheumatoid arthritis and chronic viral hepatitis (García-Monzón et al., 1990; Laffon et al., 1991), suggesting that inflammatory cytokines may be involved in CD69 expression. In this context, it has been reported that a large amount of TNF-α is produced by human hepatocytes in chronic viral hepatitis (González-Amaro et al., 1994). Thus, TNF-α and other cytokines may play a key regulatory role in the inducible expression of the CD69 molecule.

Northern blot analysis demonstrated that the constitutive and inducible expression of the CD69 molecule is regulated at the transcriptional level (Hamann et al., 1993, López-Cabrera et al., 1993; Ziegler et al., 1994). To determine the molecular basis for the pattern of CD69 expression, we have isolated the 5′-region of the CD69 gene and analyzed its inducible promoter activity. We describe herein that the upstream sequence of the CD69 antigen and the promoter activity of the 5′-region of the CD69 gene. The presence of NF-κB motifs within the proximal promoter region of the CD69 gene may account for the TNF-α-inducible promoter activity.

### EXPERIMENTAL PROCEDURES

Isolation of Genomic Clones Containing the 5′-Region of the CD69 Gene—A total of 5×106 clones from an EcoRI-digested human chromosome 12-specific library in Charon 21A (ATCC 57727) were screened as described (Santí’s et al., 1994) using a 456-bp 5′-labeled probe from the 5′-end of the CD69 cDNA (nucleotides 12-467) (López-Cabrera et al., 1993). After four rounds of screening, three positive clones were isolated. These clones were then hybridized with 32P-labeled oligonucleotides derived from the 5′-translated (nucleotides 12-31) and transcribed sequences of the CD69 cDNA (López-Cabrera et al., 1993). Since these clones hybridized only with the oligonucleotide derived from the 5′-translated sequence, they contained DNA inserts corresponding to the 5′-portion of the CD69 gene.

Restriction analysis of the clones indicated that they possessed the same 3-kbp EcoRI insert, which was subcloned into the pBluescript plasmid (Stratagene, La Jolla, CA). DNA sequencing was performed by the dideoxy termination method either by subcloning restriction fragments into pBluescript vectors or by direct oligonucleotide-primed DNA sequencing with internal primers.

Plasmid Constructions, Transfections, and Luciferase Assays—Restriction fragment sequence data and partial DNA sequencing of the cloned 3-kbp insert revealed that it contained the first exon, a part of the first intron, and a 2.1-kbp fragment containing the putative upstream regulatory region of the CD69 gene. Five restriction fragments of the latter region (EcoRI-Sad, PvuII-Sad, HindIII-Sad, BglII-Sad, and XbaI-Sad of 2.1, 1.4, 0.64, 0.49, and 0.27 kbp, respectively) were inserted downstream of the luciferase gene in pSV2-neo (Promega, Madison, WI), which contains the cytomegalovirus promoter ligated to the β-galactosidase gene, in each transfection. After 48 h of transfection, luciferase activity was determined in cell extracts containing identical β-galactosidase activity according to the instructions of the luciferase assay kit (Promega). Light emission was measured in a Lumat LB9501 luminometer (Berthold, Wildbad, Germany), and the results are expressed as relative light units. To analyze the effect of PMA and TNF-α on the CD69 promoter activity, half of the transfected cells were treated for 12-16 h with either 20 ng/ml PMA or 50 ng/ml human recombinant TNF-α (3.2×107 units/ml; Wichem, Vienna). As positive controls for PMA and TNF-α stimulation, we used the plasmids pGL2 (Promega), in which the luciferase gene is under the control of the SV40 early promoter, and pKB-F-Luc (a gift from Dr. A. Israel, Institut Pasteur, Paris), which contains a trimer of the NF-κB motif of the H-2Kk gene (Yano et al., 1987) upstream of the herpes simplex virus thymidine kinase gene promoter.

To obtain K562 stable transfectants, cells were cotransfected by electroporation with 50 μg of pAIM1.4-Luc and 10 μg of pSV2-neo as described previously (Nueda et al., 1993), selected in the presence of G418 (1 mg/ml), and cloned by limiting dilution. To analyze the activation of the CD69 promoter by NF-κB/Rel family members, K562 cells were cotransfected, in the presence of 20 μg of Lipofectin, with 5 μg of CD69 promoter-derived plasmids and 5-10 μg of the expression vector pRC/CMV-p50, pRC/CMV-c-Rel, or pRC/CMV-RelA (kindly provided by Dr. A. Israel), which contained the full-length cDNA encoding each protein, into the pRC/CMV plasmid (Invitrogen, San Diego, CA) (Le Bail et al., 1993).

Fluorescence-activated Cell Sorting Analysis—K562 cells were treated for 16 h with either PMA (20 ng/ml) or TNF-α (50 ng/ml), and surface expression of the CD69 antigen was analyzed by flow cytometry using mAb TP1.55 (Cebrián et al., 1988; Sánchez-Mateos and Sánchez-Madrid, 1991). Cells were incubated at 4°C with 100 μl of hybridoma culture supernatant, followed by washing and labeling with a fluorescein isothiocyanate-tagged goat anti-mouse Ig (Pierce). Cell-surface fluorescence was analyzed using a FACScan flow cytometer (Becton-Dickinson & Co, Mountain View, CA).

Nuclear Extracts and Electrophoretic Mobility Shift Assays—Cos-7 cells were cotransfected by the calcium phosphate precipitation procedure with the expression vectors pRC/CMV-p50 (0.5 μg/ml) and pRC/CMV-RelA (1.0 μg/ml). Small-scale nuclear extracts from these cells and untransfected K562 cells were prepared according to a procedure described elsewhere (Schreiber et al., 1989). Nuclear extracts from stimulated K562 cells were obtained after a 6-h treatment with PMA (20 ng/ml) or human recombinant TNF-α (50 ng/ml).

Binding reactions for gel retardation assays were performed at 0°C in a volume of 20 μl containing 10 μg Hepes, pH 7.6, 10% glycerol, 50 μM KCl, 6 μM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 5 μg of poly(dI-dC), 0.5 ng of 3′-labeled probe, and 2 μg of nuclear extract. After the binding reaction, the mixtures were electrophoretically separated on 4–5% nondenaturing polyacrylamide gels. When indicated, 0.5 μl of rabbit anti-p50 (Kieran et al., 1990), anti-RelA, anti-c-Rel, or anti-p52 specific polyclonal antibodies were added to the corresponding binding reaction prior to the addition of the radiolabeled probe. These antibodies were kindly provided by Drs. A. Israel and Dr. R. B. Kieran. For competition, a 50-fold molar excess of unlabelled oligonucleotide was added to the binding reaction prior to the addition of the probe. The sequences of the oligonucleotide probes (and their complementary sequences) used in this study were as follows: CD69-81-bp, 5′-GATCAGAACAAGGGAAAACCCATCTCTC-3′ (nucleotides 170 to 189); CD69-100-bp, 5′-GATCAGAACAAGGGAAAACCCATCTCTC-3′ (nucleotides 232 to 260). The synthetic oligonucleotides used for competition were as follows: VP9, 5′-CCCTGGTGTCCCCCTGAGGTTCTCCTC-3′ (a gift from Dr. J. M. Redondo, Hospital de la Princesa, Madrid), containing the two NF-κB-binding sites from the vascular cell adhesion molecule-1 promoter; KBF, 5′-AGCCTGGGTAGCCCTCCAT-3′; and OCT-1, 5′-GCTGTTGAGCCTCCTCCATGAAGACTC-3′.

### RESULTS

Structure of the 5′-Region of the CD69 Gene—The gene encoding the human CD69 antigen has previously been mapped to chromosome 12p13-p12 (López-Cabrera et al., 1993). To isolate the 5′-regulatory region of the CD69 gene, an EcoRI-digested chromosome 12-specific library was screened with a 456-bp specific probe that contained the 5′-end of CD69-encoding cDNA. Three positive clones containing the same 3-kbp insert were obtained. Restriction mapping analysis and hybridization...
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Functional analysis of the CD69 promoter. The schematic representation of the CD69 promoter-based reporter gene constructs is shown on the left, and the upstream region of the CD69 gene is represented at the top. The position of the first exon and the first intron are indicated by filled and hatched boxes, respectively. The nomenclature of the deletion plasmids is based on the most 5'-nucleotide of the CD69 gene sequence present, and its position is denoted relative to the transcription initiation site (position +1). The basal and PMA-inducible promoter activity of the 5'-region of the CD69 gene was determined by transient expression of luciferase gene-based constructs in K562 cells. Each transfection was carried out at least four times, and the data from a representative experiment are shown on the right. INDUC., induction.
plasmid pAIM1.4-Luc, and the TNF-α-induced luciferase activity was analyzed in three independent clones. The luciferase activities were represented as-fold activation over the unstimulated construct pAIM1.4-Luc cotransfected with 10 μg of empty vector pRc/CMV. Results shown are representative of four experiments.

(Fig. 1B) that could be recognized by the immediate-early growth-response transcription factor Egr-1/Krox-24, which has been described to be inducible by PMA (Krämer et al., 1994).

TNF-α Treatment induces the expression of the CD69 antigen and the activity of the CD69 Gene Promoter—The restricted expression of the CD69 antigen in vivo, at places where inflammation occurs (García-Monzón et al., 1990; Lafón et al., 1991), and the presence of three putative NF-κB-binding sites within the CD69 promoter region led us to study the effect of TNF-α, a cytokine that promotes inflammation and induces NF-κB (Baueuerle, 1991; Baueuerle and Henkel, 1994), on the expression of the CD69 gene. First, we analyzed by flow cytometry the surface expression of the CD69 antigen on unstimulated K562 cells and on K562 cells treated for 16 h with PMA (20 μg/ml) or TNF-α (50 ng/ml). Staining of cells with myeloma P3X63 antibody was included as a negative control. B, K562 cells were stably transfected with plasmid pAIM1.4-Luc, and the TNF-α-induced luciferase activity was analyzed in three independent clones. The luciferase activities were represented as-fold activation over the unstimulated construct pAIM1.4-Luc were cotransfected into K562 cells with 5 or 10 μg of expression vector pRc/CMV-p50, pRc/CMV-RelA, or pRc/CMV-c-Rel. The luciferase activities were represented as-fold activation over the unstimulated construct pAIM1.4-Luc cotransfected with 10 μg of empty vector pRc/CMV. Results shown are representative of four experiments.

The kB-2 Site Is Responsible for TNF-α Inducibility of the CD69 Gene Promoter—To identify the cis-acting sequences of the CD69 gene promoter involved in the response to TNF-α, K562 cells were transiently transfected with the different CD69 promoter-derived constructs, which contained three, two, or none NF-κB motifs of the promoter (Figs. 1B and 2). Comparison of the luciferase activity produced by the different plasmids in unstimulated and TNF-α-stimulated cells showed that deletion of the sequences located between positions –480 and –255, which eliminated the kB-3 site, did not significantly affect the response to TNF-α, whereas further removal of the sequences from positions –255 to –170, which deleted the kB-2 site, abolished the induction by TNF-α (Fig. 4A). It is interesting to note that this unresponsive construct (pAIM170-Luc) still contained the kB-1 site. These results indicate that the response to TNF-α is mediated, at least in part, by the binding of NF-κB/Rel-related proteins to the kB-2 site.

To confirm this point, K562 cells were cotransfected with the CD69 promoter constructs and the expression vector pRc/CMV-RelA. As shown in Fig. 4B, the pattern of transactivation of the different CD69 promoter fragments by RelA correlated with the response of these constructs to TNF-α (Fig. 4A). Therefore, deletion of the sequences containing the kB-2 site greatly diminishes the transactivation of the CD69 promoter by RelA.

Members of the NF-κB/Rel Family Bind to the kB-2 and kB-1 motifs of the CD69 Gene—The data presented above indicate that the TNF-α-induced expression of the CD69 gene is mainly mediated by the kB-2 site. However, since the shortest promoter fragment responsive to TNF-α (pAIM255-Luc) contained the kB-2 and kB-1 sites, the contribution of the latter NF-κB motif to the overall TNF-α response cannot be ruled out. To analyze whether these two putative NF-κB-binding sites of the CD69 promoter were capable of binding NF-κB/Rel family members, gel retardation assays were performed using probes double-stranded oligonucleotides (CD69-xB-1 and CD69-xB-2) containing these motifs. First, these probes were incubated with nuclear extracts from COS-7 cells cotransfected with the expression vectors pRc/CMV-p50 and pRc/CMV-RelA. Two major DNA-protein complexes were observed with both oligonucleotides when they were incubated with nuclear extracts from transfected COS-7 cells (Fig. 5A). These bands
corresponded to the homodimer of p50 (KBF1) and the heterodimer p50/RelA (NF-κB) and were not observed using extracts from untransfected cells (Fig. 5A, lane 1). Competition assays with an excess of oligonucleotide KBF, which contains the NF-κB motif of the H-2Kb gene, completely prevented the formation of the two DNA-protein complexes. In contrast, the addition of an excess of the unrelated oligonucleotides AP-1 and OCT-1 did not compete the binding to both probes (Fig. 5A).

To characterize the NF-κB/Rel-related proteins that bind to the NF-κB sites of the CD69 promoter in CD69-expressing cells, the two oligonucleotide probes were incubated with nuclear extracts prepared from untreated K562 cells and from K562 cells treated with either PMA or TNF-α. Four inducible DNA-protein complexes (a-d) were observed with both oligonucleotide probes when they were incubated with extracts from PMA- and TNF-α-treated K562 cells (Fig. 5B). The specific DNA-protein complexes observed with both probes displayed identical electrophoretic mobility, suggesting that they bind the same nuclear factors. It is interesting to note that oligonucleotide CD69-κB-1, which binds a lesser amount of nuclear proteins, contained one mismatch with respect to the consensus NF-κB-binding site (5′-GGGNNYYC-3′), whereas oligonucleotide CD69-κB-2 perfectly matched the consensus sequence (Baeuerle, 1991). In both cases, the addition of an excess of unlabeled specific oligonucleotide to the binding reaction completely abolished the formation of the inducible DNA-protein complexes (Fig. 5B). Similarly, an equal amount of the heterologous oligonucleotide VP9, which contained two NF-κB-binding sites from the vascular cell adhesion molecule-1 gene promoter, efficiently competed the specific complexes (Fig. 5B). In contrast, the formation of these DNA-protein complexes was not blocked by the addition of a heterologous competitor that lacked NF-κB binding sequences (Fig. 5B).

To identify the nature of the NF-κB/Rel family members that bind to the NF-κB motifs of the CD69 gene promoter, the binding reactions were preincubated with antiserum specific to p50, RelA, c-Rel, or p52 (Fig. 5C). The anti-p50 antiserum induced the disappearance or reduced the intensity of complexes b–d. The anti-RelA antiserum blocked the formation of complexes a and b, whereas the anti-c-Rel antiserum inhibited only complex c. In contrast, the anti-p52 antiserum did not interfere with the formation of any of these complexes. These results indicate that the slower migrating complex (complex a) corresponds to a homodimer of RelA, which is more easily detected when using oligonucleotide CD69-κB-2. Then, from top to bottom, the complexes correspond to p50/RelA (complex b) and p50/c-Rel (complex c) heterodimers and to a p50 homodimer (complex d). Taken together, these results confirm that the NF-κB motifs of the proximal CD69 promoter are capable of binding to inducible NF-κB/Rel factors, and therefore, they may be involved in the TNF-α-mediated induction of CD69 gene expression.

**DISCUSSION**

The transcription of the CD69 gene appears to be tightly regulated in vivo, as it is almost exclusively expressed at sites where inflammation takes place, suggesting that inflammatory cytokines may participate in the control of the expression of this gene. In this report, we describe the isolation and functional characterization of the human CD69 gene promoter region. We have focused mainly on the identification of the cis-acting sequences and the nuclear factors involved in the inducible expression of the CD69-encoding gene.

Deletion analysis of the 5′-flanking region of the CD69 gene has allowed the identification of a proximal fragment of 94 bp (nucleotides –78 to +16) that is sufficient to govern basal and PMA-induced promoter activity. This proximal promoter domain contains a canonical TATA box and a GC-rich sequence that could be a target for Sp1, a ubiquitous and constitutive transcription factor that binds to the promoter of many genes, and Egr-1/Krox-24, a zinc-finger transcription factor whose expression can be induced by various agents, including phorbol esters, and that has been implicated in the activation of T- and B-lymphocytes (McMahon and Monroe, 1995; Krämer et al., 1994; Pérez-Castillo et al., 1993). Since the synthesis of CD69-encoding mRNA is rapidly induced upon PMA stimulation of
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Fig. 5. The \( \kappa B \)-2 and \( \kappa B \)-1 motifs of the proximal CD69 promoter region are recognized by NF-\( \kappa B \)/Rel family members. A mobility band shift assays were performed with oligonucleotide probes CD69\(-\kappa B\)-1 (lanes 1–5) and CD69\(-\kappa B\)-2 (lanes 6–9), which contained the putative NF-\( \kappa B \) motifs located at positions \(-160\) and \(-223\), respectively. Nuclear extracts from COS-7 cells (lane 1) and from COS-7 cells transfected with pRcCMV-p50 and pRcCMV-RelA (lanes 2–9) were incubated with 0.5 ng of each double-stranded oligonucleotide probe. Competitor oligonucleotides were added at 50-fold molar excess and included oligonucleotide KBF, which contains the NF-\( \kappa B \) motif of the promoter of the H-2K\( ^{b} \) gene (lanes 3 and 7) and the unrelated oligonucleotides OCT-1 (lanes 4 and 8) and AP-1 (lanes 5 and 9). B, oligonucleotide probes CD69\(-\kappa B\)-2 (lanes 1–6) and CD69\(-\kappa B\)-1 (lanes 7–12) were incubated with nuclear extracts from uninduced K562 cells (lanes 1 and 7) and from K562 cells stimulated with PMA (P; lanes 2 and 8) or TNF-\( \alpha \) (lanes 3–6 and 9–12). Competitor oligonucleotides were added at 50-fold molar excess and included the specific competitors (lanes 5 and 11), oligonucleotide VP9 (which contains two NF-\( \kappa B \) motifs of the CD69 promoter (lanes 1–6)), RelA (lanes 2–6), c-Rel (lanes 7–12), and p50/c-Rel heterodimers and a p50 homodimer. The most prominent complexes were composed of p50/RelA and p50/c-Rel heterodimers. The detection of RelA homodimer binding to the \( \kappa B \)-2 motif, a complex that is not easily detected with the NF-\( \kappa B \)-8 sites of other promoters (Ganchi et al., 1993), is consistent with the observation that the sequence of this motif matches perfectly with the consensus binding sequence for RelA, 5'-GGGRNTTCC-3' (Kunsch et al., 1992).

We have recently demonstrated that the expression of the CD69 gene is regulated at the post-transcriptional level by a rapid degradation pathway associated with AU-rich sequence motifs (Santís et al., 1995). This mechanism of regulation has
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been found in many genes involved in inflammatory and activation responses. Most of these genes code for cytokines and oncoproteins, which are implicated in the initial events leading to activation and proliferation of the cells. In addition, the transcription of these genes appears to be highly regulated by the NF-κB motifs of their promoters (Baeuerle, 1991; Baeuerle and Henkel, 1994). Therefore, our findings, which highlight the functional relevance of the NF-κB motifs of the CD69 gene, further support the idea regarding a general mechanism involved in the control of expression of activation-associated genes during the early phase of the immune response.

The expression of CD69-encoding mRNA can be easily induced in most leukocytes by treatment “in vitro” with a wide range of stimuli such as PMA or anti-CD3/T cell receptor mAb (López-Cabrera et al., 1993; Ziegler et al., 1994). In addition, the expression of CD69 on activated T-lymphocytes from cell infiltrates of various chronic inflammatory diseases such as rheumatoid arthritis and chronic viral hepatitis has been documented (García-Monzón et al., 1990; Laffon et al., 1991). It is also known that TNF-α is an important inflammatory mediator that is actively secreted by several cell types at inflammatory sites (González-Amaro et al., 1994; Vassalli, 1992). Our findings strongly suggest that the pro-inflammatory cytokine TNFα may play a key role “in vivo” in the expression of CD69 by inflammatory cells. Interestingly enough, we have previously described that CD69 antigen is in turn capable of generating signals that induce the synthesis of TNF-α by lymphoid cells (Santí’s et al., 1992). Thus, it is feasible that, at sites of inflammation, a positive feed-back loop is established between the expression of CD69 and the production of TNF-α. The putative ligand of CD69 should be clearly involved in this condition. The above phenomenon could have an important role in the perpetuation of several inflammatory diseases and could be related to the resistance of some patients with these conditions toward the current anti-inflammatory therapy.

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