THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 268, No. 2, Issue of January 15, pp. 1355-1361, 1993
Printed in U.S.A.

Involvement of Alu Sequences in the Cell-specific Regulation of Transcription of the γ Chain of Fc and T Cell Receptors*

(Received for publication, August 18, 1992)

Anna T. Brini‡, Gai M. Lee, and Jean-Pierre Kinet

From the Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852

The FcεRI-γ chains are expressed in a variety of hematopoietic cells where they play a critical role in signal transduction. They are part of the high affinity IgE receptor in mast cells, basophils, Langerhans cells, and possibly other cells; a component of the low affinity receptor for IgG (FcγRIIIA or CD16) in natural killer cells and macrophages; and part of the T cell antigen receptor in subsets of T cells. Here we have investigated the transcriptional regulation of the γ chain gene by analyzing the 2.5-kilobase sequence upstream of the transcription start site. This sequence contains a promoter specific to cells of hematopoietic lineage. However, the tissue specificity of this promoter is only partial because it is active in all of the hematopoietic cells tested here, regardless of whether they constitutively express FcεRI- γ chain transcripts. We have identified two adjacent cis-acting regulatory elements, both of which are part of an Alu repeat. The first (−445/−366) is a positive element active in both basophils and T cells. The second (−365/−264) binds to nuclear factors, which appear to be different in basophils and T cells, and acts as a negative element in basophils and as a positive one in T cells. Thus, this Alu repeat (90% identical to Alu consensus sequences) has evolved to become both a positive and negative regulator.

The γ chains initially described as subunits of the high affinity IgE receptor (FcεRI)[1] are expressed in various hematopoietic cells and are part of several receptor multimeric complexes (1-3). Homodimers of γ chains, together with an α and a β chain, form the tetrameric FcεRI receptor in mast cells, basophils, and Langerhans cells (4-6). The same γγ homodimers also associate with the low affinity receptor for IgG (FcγRIII, CD16) in mast cells, basophils, and monocytes (7). In addition to forming disulfide-linked homodimers, the γ chains interact with two other homologous proteins, the ζ and η chains of the T cell antigen receptor. These three chains generate six different dimers, the γγ, ζζ, and ηη homodimers and the γζ, γη, and ζη heterodimers, all of which associate with the T cell antigen receptor in some subsets of T lymphocytes (8-10). The γγ, ζζ homodimers and the γζ heterodimers are also part of the low affinity IgG receptor (CD16-FcγRIII) in natural killer cells (11-15).

The γ, ζ, and η chains belong to the same family of molecules. Their respective genes show a similar genomic organization and are located on mouse and human chromosome 1 (16-23). Furthermore, they contain homologous amino acid motifs which appear to be critical to the initiation of receptor-mediated signal transduction (3).

Previously, our laboratory has characterized cDNA and genomic clones for the human FcεRI-γ chain (16). The gene consists of five exons and spans 4 kilobases. There is a major start site localized 25 base pairs upstream of the start codon, but a few additional start sites are also present. The gene does not show a typical TATA box but contains inverted CAAT and GC boxes at positions −199 and −135, respectively. This gene is constitutively expressed in certain cell types such as basophils (1) but is only expressed in subsets of others cells such as T lymphocytes (8-10).

In this study, we have characterized the 5′ region and the promoter of the FcεRI-γ gene and have detected the presence of positive and negative cis-acting elements. We show that the promoter is hematopoietic-specific and that Alu sequences play a role in the positive and negative regulation of transcription.

EXPERIMENTAL PROCEDURES

Sequencing the 5′-Flanking Region of the Human FcεRI-γ Gene—A genomic clone longer than the one previously described and sequenced (16) was isolated. A 5-kilobase *Sal*I fragment was subcloned into the pBS± vector (Stratagene), and a restriction map was established. A 2.2-kilobase *Hin*dIII/*Bgl*II fragment corresponding to the 5′ sequence of the gene was isolated, subcloned, and sequenced by the dideoxy chain termination method using 21-base pair unlabeled oligonucleotide primers as described before (16).

Plasmid Construction—DNA from a genomic clone was used as the template in a polymerase chain reaction to generate various fragments of the 5′-flanking region of the FcεRI-γ gene. Oligonucleotides (24-mer) starting at positions −445, −295, −237, −161, −95, and −54 were chosen as 5′ primer, and the 3′ primer was the same oligonucleotide ending at position +26. Convenient restriction sites were added to each oligonucleotide. The polymerase chain reactions were performed according to standard procedures in a 100-μl reaction volume containing 1.5 mM $MgCl_2$, 50 pmol of primers, 1 unit of Taq polymerase, and 200 μM dNTPs. The polymerase chain reaction products were subcloned into the *Hin*dIII and *Xba*I sites of the pCAT-basic vector (Promega).

The −2382/+26 construct was generated by ligation of the −2382/−180 restricted fragment into *Hin*dIII and *Bgl*II sites of the −445 construct. The −1631, −1296, and −365 CAT constructs were generated by excising the *Hin*dIII and *Cla*I, *Hin*dIII and *Ppu*MI, *Hin*dIII, and *Acc*I fragments respectively, from the −2382 construct prior to religation.

The −445 gap (−365/−264) CAT construct was obtained by a two-

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L03533.

‡ To whom correspondence should be sent: 12441 Parklawn Dr., Twinbrook II, Rm. 108, Rockville, MD 20852.

[1] The abbreviations used are: FcεRI, high affinity receptor for immunoglobulin E; CAT, chloramphenicol acetyltransferase; μF, microfarads; EMSA, electrophoretic mobility shift assay; RBL, rat basophilic leukemia; Alu repeat, major interdisperse repetitive nucleotide sequence in the mammalian group.

step polymerase chain reaction. The −445/−264 (*Hin*dIII/*Acc*I) fragment was inserted upstream (*Bgl*II) or downstream (*Bam*HI) of the SV40 promoter in the pCAT-promoter vector (Promega). Standard procedures were used in all plasmid constructions (24). Both strands of each constructs were verified by dideoxy sequencing as above.

Cells—The CEM-CM-3, Jurkat, HeLa, COS-7, U937, HL-60 cell lines were from the American Type Culture Collection. The human basophilic KU812 cell line was a gift from Dr. K. Kishi (25); the Daudi cell line was from Dr. M. Schwabe NCI-Frederick, MD; the NK 3.3 cell line was a gift from Dr. J. Kornbluth (26). These cells were propagated in the recommended media, supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, and antibiotics in a humidified atmosphere of 5% CO_2 and 95% air. RBL-2H3 cells were maintained as described previously (27).

Transfection—Cells growing in suspension were transfected while in exponential growth, whereas adherent cells were transfected when 70–80% confluent. DNA was transfected into HeLa, U937, and COS-7 cells by the DEAE-dextran method (24, 28). The other cell lines were transfected by electroporation using a Bio-Rad Gene Pulser apparatus. 500 μl of the various cells in complete medium was electroporated using the following conditions: 5×10^6 Jurkat cells, 300 volts and 500 μF; 5×10^6 CEM-CM-3 cells, 200 volts and 960 μF; 1×10^7 RBL-2H3 cells, 250 volts and 500 μF; 1×10^7 KU812 cells, 330 volts and 960 μF. Cotransfection of pCMV-β-galactosidase plasmid (29), which comprises an *Escherichia coli LacZ* gene driven by the cytomegalovirus promoter, was used as a control and to determine the best conditions for transfection. Cells were harvested 24 h after transfection, washed twice with cold phosphate buffered saline, and lysed by repeated cycles of freeze-thawing in 250 mM Tris (pH 7.9).

Enzyme Assays—The β-galactosidase assay was performed as described (24) with 10–30 μg of protein. The protein concentration was determined by the Bradford method (30). The CAT activity in cell lysates, normalized for protein concentrations and for unit of β-galactosidase, was determined by a slight modification of the method described by Gorman *et al.* (31). In short, the acetylated chloramphenicol was separated from the nonacetylated form by thin-layer chromatography and developed in chloroform/methanol (95:5, v/v). The radioactivity of each acetylated and nonacetylated spot was directly determined with the Ambis radioanalytic imaging system. CAT activity is expressed as acetylated forms/total × 100/100 units of β-galactosidase. Every transfection experiment was performed in duplicate. The results are expressed as the means (± S.E. or S.D as indicated) of at least three different transfections for each construct and for each cell line.

Nuclear Extract Preparation—Nuclear extracts were prepared as outlined by Dignam *et al.* (32). Buffers A, C, and D contained 0.5 mM phenylmethylsulfonyl fluoride and 0.5 mM dithiothreitol to minimize proteolysis. Cells were incubated on ice in 5 volumes of buffer A. After 10 min cell membranes were disrupted by 12 strokes of a Dounce homogenizer (pestle B), and the intact nuclei were pelleted for 20 min in a microcentrifuge at 4 °C. The nuclei were broken by 10 strokes of the Dounce homogenizer in 2 volumes of buffer C and then stirred for 30 min at 4 °C. The nuclear extract was then clarified by centrifugation (as above) and dialyzed against buffer D for 2 h. The total protein concentration was determined by the Bradford method (30). Aliquots of nuclear extract were used immediately or stored at −80 °C for later use.

Electrophoretic Mobility Shift Assay (EMSAs)—Electrophoretic mobility shift assays were performed as described (33) with modifications. The double-stranded DNA fragments spanning the sequences −445/−180, −445/−264, and −445/−365 of the γ gene were generated by enzymatic digestion and isolated by standard techniques (24). The fragments were end labeled using [α-^{32}P]ATP and [α-^{32}P]GTP (3000 Ci/mmol; Amersham Corp.) by Klenow-large fragment polymerase. The probe, spanning the region −366/−290 was generated by synthesizing two complementary 77-mer oligonucleotides and by annealing the two oligonucleotides at an equimolar ratio in the presence of 150 mM NaCl. The resultant double-stranded synthetic oligonucleotide was gel purified on a 12% nondenaturing polyacrylamide gel and subsequently end labeled with [γ-^{32}P]ATP and T4 kinase. All probes were purified twice over a Sephadex G-25 column. The unlabeled double-stranded oligonucleotides used for competition experiments, including those corresponding to the binding sites for NF-kB (34) and AP-1 (35), were prepared and purified as described above. The poly(dA)·poly(dT) double-stranded oligonucleotide was purchased from Pharmacia LKB Biotechnology Inc.

Binding assays were carried out in a binding buffer containing 20 mM Hepes (pH 7.8), 60 mM KCl, 0.2 mM EDTA, 1 mM dithiothreitol, 1 mM $MgCl_2$, 5% glycerol, about 0.5–1 ng of ^{32}P-labeled DNA fragment (2×10^4 cpm), 1 or 2 μg of poly(dI·dC) or poly(dG·dC) (Pharmacia), as indicated, and 2–30 μg of nuclear extract in a total volume of 20 μl. Since the −366/−290 sequence is an AT-rich region and contains 23 adenines, we performed EMSA with this probe using two different nonspecific polyanion competitors (poly(dI·dC) and poly(dG·dC)) to unmask complexes which would be undetectable only in the presence of poly(dI·dC) (36, 37). For competition experiments, the 100- or 200-fold molar excess of unlabeled competitor was added to the binding reaction 5 min before the radiolabeled probe. Extracts and probes were incubated for 20 min at room temperature and then analyzed either on a 5 or a 8% polyacrylamide nondenaturing 0.5 × TBE gel (acrylamide:bisacrylamide ratio of 38:2) depending on the length of the probes. Gels were dried and exposed to Kodak XAR-2 film without intensifying screens for a few hours or overnight.

RESULTS AND DISCUSSION

Consensus Sequences for cis-Acting Elements in the 5′-Flanking Region of the Human FcεRI-γ Gene—As a first step in the characterization of the regulatory sequences involved in the transcriptional control of the human FcεRI-γ chain gene, we completed the sequencing of the 5′-flanking region of the gene from a previously described genomic clone (Fig. 1*A*). This allowed us to localize a number of consensus sequences for potential cis-acting regulatory elements (Fig. 1*B*), among which are mast-cell specific elements (GATA), T cell-specific elements (Lyf-1, CTF-1), SP-1 sites, an inverted CAAT box, several γ-interferon- and six interleukin-6-responsive elements (38). The γ-interferon elements are likely involved in the up-regulation of the FcεRI-γ mRNA levels induced by γ-interferon in purified monocytes and in the U937 cell line.[2] In addition, we identified two Alu repeats, one between −2131 and −2358 and another between −313 and −582 from the ATG start codon (see also below).

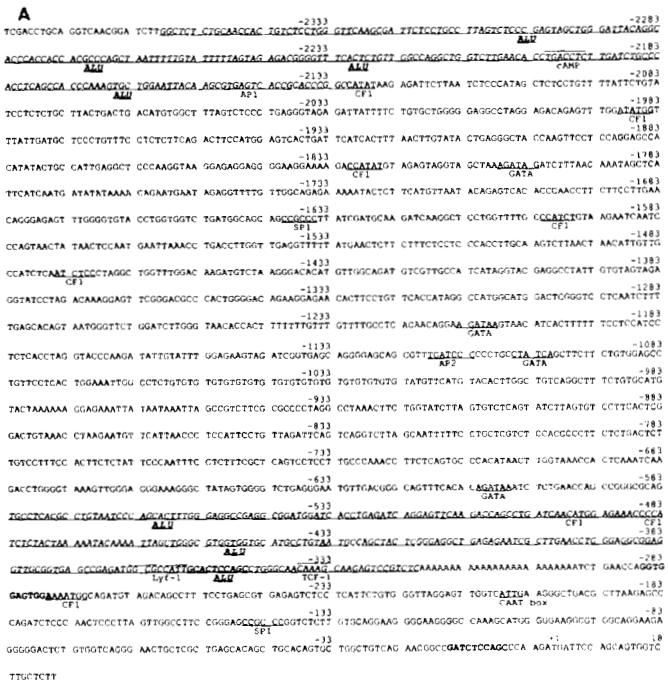
Identification of a Hematopoietic-specific Promoter—To identify the important regulatory elements of the 5′-flanking region of the gene, we inserted various fragments in an expression vector immediately upstream of the chloramphenicol acetyltransferase (CAT) gene and analyzed the effect of the various deletions on the transcription of the CAT gene in transfected cells. Cotransfection of a pCMV-β-galactosidase plasmid with the CAT constructs allowed the systematic monitoring of the transfection efficiency by enzymatic assay.

The rationale of the different deletion constructs shown in Fig. 2*A* is as follows. The −95 construct ends 3′ of an SP-1 site, the −161 construct contains this SP-1 site, the −237 construct contains the inverted CAAT box, and the −295 construct ends 3′ of a stretch of 23 consecutive adenines. The remaining constructs were generated from convenient enzymatic restriction sites.

The analysis of the relative CAT activity in rat basophilic leukemia cells (RBL-2H3), in the human basophilic cell line KU812, and in non-hematopoietic HeLa cells is shown in Fig. 2*B*. A similar analysis in T cells (the CEM-CM-3 and the Jurkat cell lines) and HeLa cells is shown in Fig. 2*C*. No activity is detected in transfected HeLa cells, regardless of the construct and despite a high level of transfection efficiency. By contrast, the −2382 construct shows a substantial activity in basophils (7.4%) and a basal activity in T cells (1.7%). However, the −1637 construct has no activity in either cell type with the possible exception of KU812 cells, in which a weak activity is detected. Furthermore, the −1296 construct shows an activity higher than the one observed for the −1637 construct, except again in KU812.

Taken together, these data suggest that the promoter is active in basophils and T cells but not in non-hematopoietic cells; that the sequence between −1296 and −1631 contains a

[2] A. Brini and J.-P. Kinet, unpublished data.



B

Putative regulatory motifs in the -2382 5'-flanking region of the FcεRI γ-chain gene

ELEMENT	#of sites	Sequences	POSITION
AP1	2	TGASTMA	-2148
			-2142
AP2	1	CCMNSSS	-1112
CF1	9	ANATGG	-2126
			-1989
			-1825
			-1596
			-1477
			-498
			-480
			-368
			-275
CTCF1	7	CCCTC	-2037
			-1906
			-1842
			-855
			-660
			-635
			-80
E2A	5	RCAGNTG	-1594
			-1428
			-270
			-47
			-40
ELP	1	CAAGGTCA	-1545
Ets-1	1	SMGGAWGY	-1324
GATA	4	WGATAR	-1807
			-1213
			-1100
			-611
H-APF-1	1	CTGGRAA	-1073
γ-IRE	17	CTKKANNY	-2326
			-2285
			-2194
			-2163
			-2087
			-2054
			-1926
			-1806
			-1616
			-1264
			-1073
			-880
			-682
			-669
			-572
			-512
Lyf-1	2	YYTGGGAGR	-402
			-557
MCBF	1	CATTCCT	-356
NF-GM	1	GRGRTTKCAY	-850
NF-IL6	6	TKNNGNAAK	-2238
			-2156
			-726
			-640
			-131
			-105
PEA3	11	AGGAAR	12
			-1961
			-1839
			-1687
			-1325
			-1217
			-1189
			-811
			-751
			-249
			-128
SP1	2	GGGCGG	-89
			-1635
TCF-1	1	MAMAG	-141
			-339

FIG. 1. **The 5′-flanking sequence of the human FcεRI-γ chain gene.** *Panel A*, nucleotide sequence of the 2,408-base pair fragment spanning the 5′-flanking region of the human FcεRI-γ chain gene. Position 1 corresponds to the start codon. Alu repeats and consensus sequences for GATA, AP-1, AP-2, CF-1, cAMP-responsive element, SP-1, and CAAT box are *underlined*. The stretches of Alu sequences are typed in *italics*, the Lyf-1 and TCF-1 binding sites are *overlined*, and the putative "γ motif" found three times in the γ promoter region is *highlighted* in **boldface**. *Panel B*, list, number, and position of the putative regulatory elements corresponding to the consensus sequences as indicated. The consensus sequences are listed in the IUPAC-IUB code (38).

negative element that down-regulates the transcription to almost undetectable levels; that the sequence between −1637 and −2382 confers a basal level of transcription in T cells and a higher level in basophils possibly because of the presence of a GATA site found at −1807.

To confirm the specificity of the promoter for hematopoietic cells we also transfected COS-7 cells, the monocytic cell line U937, and the B cell line Daudi, with the −2382 construct. No activity was detected in non-hematopoietic COS-7 cells, whereas a basal CAT activity was observed in U937 and Daudi cells (data not shown).

The Promoter Confers Only Partial Tissue Specificity—Among the hematopoietic cells chosen for transfection, the RBL-2H3, KU812, CEM-CM-3, and U937 cells express FcεRI-γ transcripts constitutively, whereas Jurkat and Daudi cells do not. However, there is no difference in the promoter activity in the transfected cells according to whether or not they express the FcεRI-γ transcripts. Therefore, the promoter analyzed here is responsible for only part of the tissue specificity (*i.e.* hematopoietic) but not for the complete specificity (*i.e.* transcription in FcεRI-γ-positive cells). It is possible that other regions of the gene may control the specificity of expression in FcεRI-γ-positive cells. More likely, gene methylation may confer that specificity as shown for the gene of the ζ chain of the T cell antigen receptor, a gene homologous to the γ gene analyzed here (39) and for other genes as well (40). Alternatively, the interaction of nuclear factors with the gene may be affected by different DNA structures in various cell types.

The −445/−295 Fragment Contains a Strongly Positive, Hematopoietic-specific Element—The most significant finding of the functional analysis with the deletion constructs is the identification of a strongly positive region between −445 and −295 (Fig. 2, *B* and *C*). This element is at least four times more potent in basophils than in T cells and is inactive in HeLa cells. It is 30 × more potent in RBL cells than the SV40 promoter and 3 × more potent in T cells (not shown). In addition, it is active in many other hematopoietic cells including U937, THP1, and HL-60, NK 3.3, murine CTLL and Daudi but not in COS-7 (not shown), confirming its specificity to cells of hematopoietic lineage. However, similar to what is observed for the full-length construct, this element is active in both FcεRI-γ-negative and FcεRI-γ-positive hematopoietic cells.

Effect of the −445/−264 Fragment on a Heterologous Promoter—To test if the −445/−264 region could also up-regulate the activity of a heterologous promoter, we inserted the −445/−264 fragment upstream or downstream of the SV40 promoter-driven CAT gene (Fig. 3*A*). The fragment has no apparent effect on the transcription of the CAT gene in transfected T cells, but it has a surprising inhibitory effect in transfected RBL cells (Fig. 3*B*). This inhibitory effect is only seen when the fragment is inserted 5′ of the CAT gene and in the forward orientation. The inhibitory effect is not seen when the insert is placed in the reverse orientation (data not shown). One possibility is that binding of basophil-specific nuclear factors to this region interferes nonspecifically with the functioning of the SV40 promoter. Another possibility is that this region contains a basophil-specific negative element (see below). In any case, the negative effect of this fragment is nullified when the downstream sequence of the homologous promoter is present. In addition, the disparate effects observed

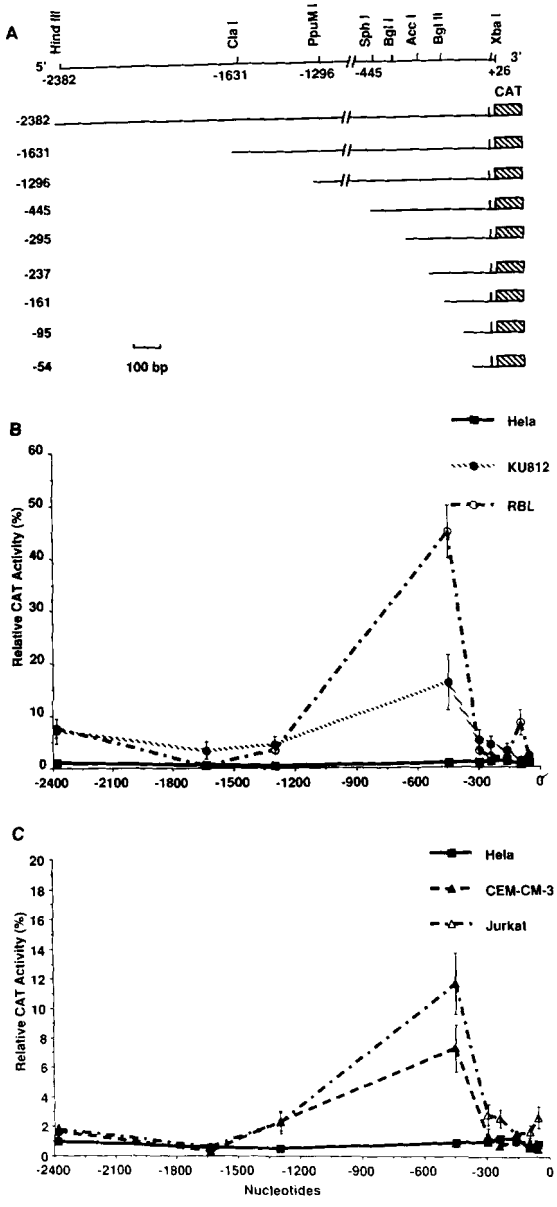


FIG. 2. **Functional analysis of the 5′-flanking sequence of the human γ chain gene by CAT assay.** *Panel A*, schematic representation of the human FcεRI-γ gene chimeric CAT plasmids. *Panel B*, CAT activities of the various deletions constructs transfected into human (KU812 cells) and rat (RBL-2H3 cells) basophils. The levels of CAT activity detected in HeLa cell lysates are also shown. *Panel C*, CAT activities in transfected human T lymphocyte cell lines (CEM-CM-3 and Jurkat) and in HeLa cells. The relative CAT activity is expressed as percent of conversion of the chloramphenicol to its acetylated forms. The graphics represent the averages of the relative CAT activity ± S.E. from at least five separate transfections for each construct and for each cell line. Each transfection was done in duplicate. The CAT activities have been standardized by using units of the β-galactosidase activity resulting from the cotransfection of the pCMV-β-galactosidase plasmid.

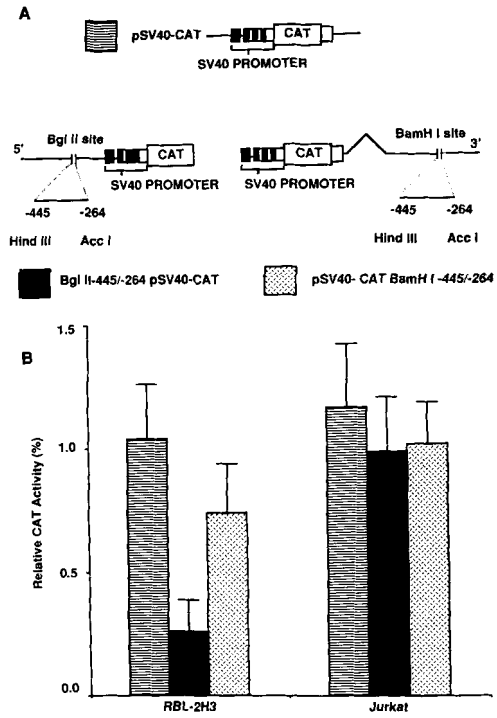


FIG. 3. **Effect of the transcriptionally active human γ sequences on a heterologous promoter.** *Panel A*, schematic representation of SV40 early promoter-CAT constructs made to test the effect of the −445/−264 γ region on a heterologous promoter. The control plasmid is the vector pSV40-CAT (▤). The −445/−264 human γ fragment was inserted 5′ (▨) or 3′ (□) of the SV40 promoter. *Panel B*, functional analysis of SV40 promoter constructs transiently transfected in RBL-2H3 cells or in Jurkat cells. The CAT activity was determined as in Fig. 2. The *bars* represent the average percent of conversion of CAT to its acetylated products ± S.D. of three separate transfections, each performed in duplicate.

in T cells and basophils support our contention that the nuclear factors interacting with this region are different in T cells and in basophils (see below).

The −445/−264 Fragment Binds to Specific Nuclear Factors—We analyzed by EMSA the capacity of the *Hin*dIII/*Acc*I fragment (−445/−264) to bind to specific nuclear factors extracted from the cells used in the functional analysis (Fig. 4). Nuclear proteins extracted from Jurkat, CEM-CM-3, and RBL cells bind to this fragment. This binding is blocked efficiently by using unlabeled −445/−180 (*lanes 4*, *9*, and *14*) or −366/−290 fragments (*lanes 5*, *10*, and *15*) but not by unrelated ones (*lanes 3*, *6*, *8*, *11*, and *13*). Therefore, nuclear factors binding specifically to the −445/−264 region are present in these cells. Furthermore, the different patterns obtained with nuclear extracts from RBL cells and from Jurkat or CEM-CM-3 cells suggest that the factors binding this region are different in T cells and basophils.

We also tested the binding capacity of the −445/−180 fragment (not shown). We observed no specific binding with a nuclear extract from HeLa cells but found virtually the same patterns of retarded bands with nuclear extracts from Jurkat, CEM-CM-3, and RBL cells as those shown in Fig. 4. This suggests that the −264/−180 region does not participate in the binding of specific nuclear factors.

The −366/−290 Region Binds to Nuclear Factors That Are Different in Basophils and T Cells—Since the −366/−290 fragment is an efficient inhibitor of the binding of nuclear factors to the −445/−264 region, we decided to use the radiolabeled −366/−290 fragment itself in binding studies (Fig. 5*A*). As expected, the patterns of retarded bands are different when the binding is performed in the presence of poly(dI·dC) or of poly(dG·dC) (36, 37). Regardless of the conditions, the patterns of retarded bands are also different in T cells (Jurkat and CEM-CM-3) and basophils (RBL-2H3 and KU812). These results are consistent with those obtained with the larger −445/−264 fragment, suggesting once again that the nuclear factors interacting with this region are different in basophils and T cells.

We subsequently studied the capacity of short, double-

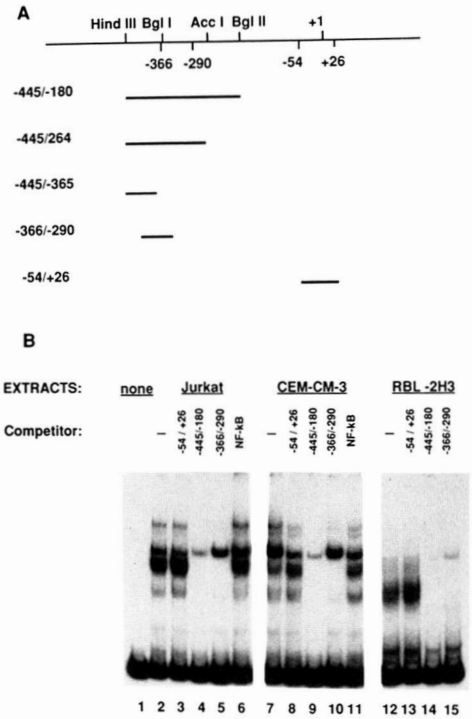


FIG. 4. **Binding of nuclear proteins to the (−445/−264) fragment of the FcεRI-γ chain gene.** Analysis was by EMSA. *Panel A*, schematic representation of probes and DNA fragments used in EMSAs. The *top* of the figure shows a partial restriction map of the region between −445 and +26. *Panel B*, EMSA performed with nuclear extracts from Jurkat cells (*lanes 2–6*), CEM-CM-3 cells (*lanes 7–11*), and RBL-2H3 cells (*lanes 12–15*). Nuclear extracts were incubated with the ^{32}P-labeled −445/−264 fragment in the presence of poly(dI·dC) as nonspecific competitor. *Lane 1* contains the labeled probe alone. Unlabeled DNA fragments used as competitors are indicated.

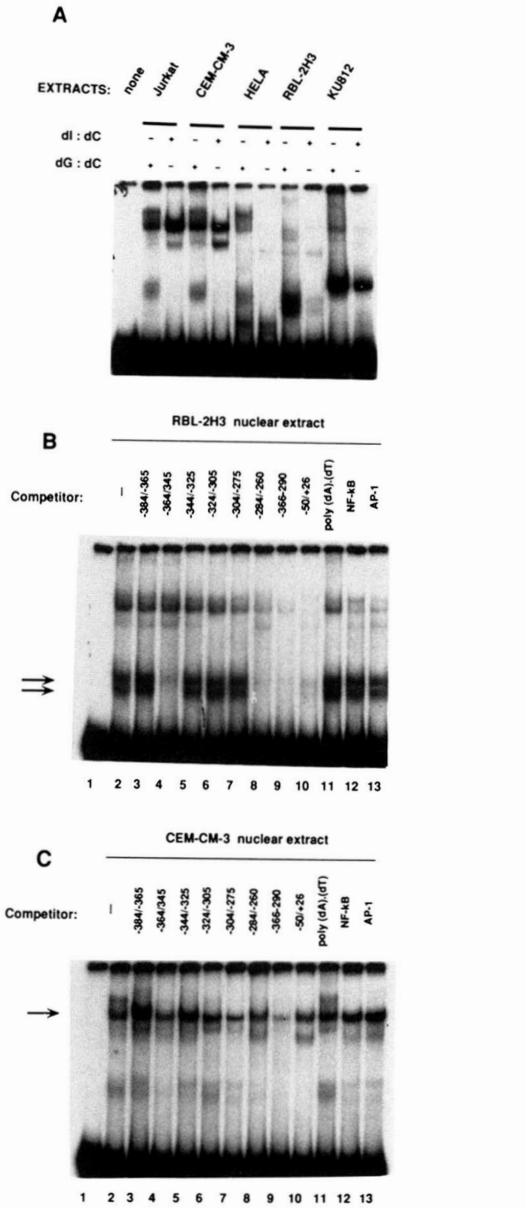


FIG. 5. **Binding of specific nuclear proteins to the −366/−290 fragment of the FcεRI-γ gene promoter.** *Panel A*, EMSA performed with nuclear extracts from Jurkat, CEM-CM-3, HeLa, RBL-2H3, and KU812 cell lines as in Fig. 4. Nuclear extracts were incubated with the ^{32}P-labeled −366/−290 fragment in the presence of poly(dI·dC) or poly(dG·dC) as nonspecific competitor. *Panels B* and *C*, EMSA performed with nuclear extracts from RBL-2H3 and CEM-CM-3 cell lines as in *panel A* and with the poly(dG·dC) as nonspecific inhibitor. DNA fragments used as competitors are indicated.

stranded oligonucleotides to inhibit specific binding to the −366/−290 element in RBL-2H3 cells (Fig. 5*B*) and in CEM-CM-3 cells (Fig. 5*C*). Two major bands in RBL cells and one major band in CEM-CM-3 cells are detected in the presence of the unlabeled NF-kB and AP-1 (*lanes 12* and *13*) and are therefore considered to be specific. These bands are not observed following competition with the homologous cold fragment (*lane 9*) but are observed following competition with the −384/−365 oligonucleotide, whose sequence is located 5′ of the relevant sequence (*lane 3*).

The −364/−345 oligonucleotide is inhibitory in RBL cells and may have a slight inhibitory effect in CEM-CM-3 cells (*lane 4*). Since a Lyf-1 motif is present in the −364/−345 region, Lyf-binding factors already known to be present in various B and T lymphocytes could potentially explain the hematopoietic specificity of the promoter. However, the surprising finding that the −284/−260 (*lane 8*) and −50/+26 (*lane 10*) oligonucleotides also inhibit binding in RBL cells but not in CEM-CM-3 cells led us to speculate that a sequence other than the Lyf-1 motif might also be involved in the binding. Indeed, sequence comparison between these two oligonucleotides and the −366/−290 element reveals a common motif if one allows for two mismatches. This motif is not found anywhere else in the 2.4 kilobases of the 5′ region. The sequence GCACTCCAGC is found at −355 right after the Lyf-1 motif, the sequence GatCTCCAGC is found at −15, and the complementary sequence to cCACTCCAcC is found at −277. Therefore, it is conceivable that the presence of this common motif explains the inhibitory effect of the −284/−260 and −50/+26 oligonucleotides in RBL cells. It is also tempting to speculate that the same basophil-specific factors interact with these three motifs. This could explain why the −445/−264 fragment has a different activity when placed in front of a heterologous promoter (see above). Obviously, further characterization of the motif and of possible binding factors needs to be done.

Detection of Two Adjacent Regulatory Elements in the −445/−264 Region—To analyze further the differential function of the −445/−264 fragment in basophils and T cells, we studied additional deletion constructs (Fig. 6). Removing the region between −365 and −264 results in an 89% increase in CAT activity in basophils when compared with the complete −445/+26 region. In contrast, no significant difference is detected

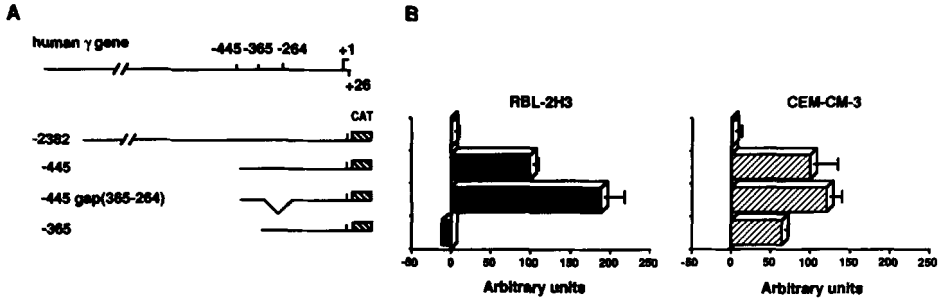


FIG. 6. **Functional analysis of the −445/−264 region: detection of two adjacent regulatory elements.** *Panel A*, schematic representation of the human FcεRI-γ gene CAT constructs. *Panel B*, CAT activities in transfected RBL-2H3 and in CEM-CM-3 cells. CAT activities were analyzed as in Fig. 2 from at least three independent transfection for each construct. The data have been normalized as follows. The activities corresponding to the −2382 and to the −445 CAT construct were considered as 1 and 100, respectively. The mean ± S.D. are shown for each construct. The differences of CAT activity between the −445 and the −445 gap (−365/−264) CAT on the one hand, and between the −445 and −366 CAT constructs on the other hand are both highly significant in RBL-2H3 cells ($p < 0.01$).

```
 -593      -583      -573      -563      -553      -543
I  AGCCGGGCGCAGTGCCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGATGGA
   :::::::::  ::: :::::::::::::::::::::::::::::::::::::::  :::
II GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGA
           10        20        30        40        50        60

 -533      -523      -513      -503      -493      -483
I  TCACCTGAGATCAGGAGTTCAAGACCAGCCTGATCAACATGGAGAAACCCCATCTCTACT
   ::::::::: :::::::::: ::::::::::: :::::::: :::::::: ::::::::
II TCACCTGAGGTCAGGAGTTCGAGACCAGCCTGGCCAACATGGTGAAACCCCGTCTCTACT
           70        80        90       100       110       120

 -473      -463      -453      -443      -433      -423
I  AAAAATAC-AAAATTAGCTGGGCGTGGTGGTGGTGCATGCCTGTAATGCCAGCTACTCGGGAG
   :::::::: ::::::::: :::::::::::  ::  :::::::  : ::::::::::::::
II AAAAATACAAAAAATTAGCCGGGCGTGGTGGCGCGCGCCTGTAGTCCCAGCTACTCGGGAG
           130       140       150       160       170       180

  -413          -403      -393      -383      -373      -363
I  GCTGA-----GAGAATCGCTTGAACCTGGGAGGCGGAGGTTGCGGTGAGCCGAGATGGCG
   ::::::.. .:::::::::::::::::: :::::::::::::::::: ::::::::::::
II GCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCGCG
           190       200       210       220       230       240

       -353      -343      -333      -323      -313      -303
I  CCATTGCACTCCAGCCTGGGCAACAAAGCAAGAGTCCGTCTCAAAAAAAAAAAAAAAAAA
   ::: :::::::::::::::::: ::: ::: ::: :::::::
II CCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTC
           250       260       270       280
```

FIG. 7. **Alignment of the cis-acting regulatory elements (*I*) with the primate-specific Alu consensus sequence (*II*).** The adjacent cis-acting regulatory elements of the γ chain gene are *underlined*. The consensus Alu sequence corresponds to the primate-specific Alu consensus sequence (41). The stretch of consecutive adenines is typical of the 3′ portion of Alu sequences, although the number of these adenines is variable. Here only 18 out of the 23 adenines found in the γ chain gene are shown. *Double dots* indicate identical nucleotides, and *dashes* correspond to gaps or deletions.

in T cells. Therefore the −365/−264 element is a negative regulator in basophils but not in T cells.

Deletion of the −445/−366 region abolishes the CAT activity in RBL cells but yield only a 40% reduction in CEM-CM-3 cells. Indeed in CEM-CM-3 cells, a further deletion of the −365/−295 region is required to reduce the CAT activity to basal level (see Fig. 2). These results indicate that the −445/−366 element is a positive element in both basophils and T cells and that the −365/−264 element contains an activator in T cells but not in basophils.

The Two cis-Acting Regulatory Elements Are Part of an Alu Repeat—As mentioned above, the 5′ sequence of the γ chain gene contains two typical Alu repeats (Fig. 1). Comparison of the sequence containing the two cis-acting regulatory elements (−445/−366 and −365/−264 elements) with Alu consensus sequences shows that the regulatory elements are within the sequence of Alu repeats. Among all subfamilies of Alu sequences (41), the best alignment was with the primate-specific Alu consensus sequence. We found that about 90% of the nucleotides are identical between the two sequences (Fig. 7).

There are examples of Alu sequences containing negative regulatory elements (42, 43), but we are not aware of any example of positive regulatory elements containing Alu sequences. Clearly the entire (−445/−366)-positive element is part of the Alu repeat. Most of the other element (−365/−264) overlaps also with the Alu consensus sequence, but we cannot exclude that the sequence from −296 to −264, which is not part of the Alu repeat, is critical for its activity. However, our binding studies suggest that the active region of the (−365/−264) element is between −365 and −290 and is within the Alu repeat.

In conclusion, we have shown that the transcription of the FcεRI-γ chain gene is regulated by positive and negative cis-acting elements. These elements are part of an Alu repeat, are only active in hematopoietic cells, and are recognized by DNA-binding proteins in a cell type-specific manner.

REFERENCES

1. Kinet, J.-P. (1990) *Curr. Opin. Immunol.* **2,** 499–505
2. Ravetch, J. V., and Kinet, J.-P. (1991) *Annu. Rev. Immunol.* **9,** 457–492
3. Kinet, J.-P. (1992) *Curr. Opin. Immunol.* **4,** 43–48
4. Blank, U., Ra, C., Miller, L., White, K., Metzger, H., and Kinet, J.-P. (1989) *Nature* **337,** 187–189
5. Wang, B., Rieger, A., Kilgus, O., Ochiai, K., Maurer, D., Fodinger, D., Kinet, J.-P., and Stingl, G. (1992) *J. Exp. Med.* **175,** 1353–1365
6. Bieber, T., de la Salle, H., Wollenberg, A., Hakimi, J., Chizzonite, R., Ring, J., Hanau, D., and de la Salle, C. (1992) *J. Exp. Med.* **175,** 1285–1290
7. Ra, C., Jouvin, M. H. E., Blank, U., and Kinet J.-P. (1989) *Nature* **341,** 752–754
8. Orloff, D. G., Ra, C., Frank, S. J., Klausner, R. D. F., and Kinet, J.-P. (1990) *Nature* **347,** 189–191
9. Vivier, E., Rochet, N., Kochan, J. P., Presky, D. H., Schlossman, S. F., and Anderson, P. (1991) *J. Immunol.* **147,** 4263–4270
10. Koyasu, S., D'Adamio, D., Arulanandam, A. R. N., Abraham, S., Clayton, L. K., and Reinherz, E. L. (1992) *J. Exp. Med.* **175,** 203–209
11. Hibbs, M. L., Selvaraj, P., Carpen, O., Springer, T. A., Küster, H., Jouvin, M. H. E., and Kinet, J.-P. (1989) *Science* **246,** 1608–1611
12. Kurosaki, T., and Ravetch, J. V. (1989) *Nature* **342,** 805–807
13. Lanier, L. L., Yu, G., and Phillips, J. H. (1989) *Nature* **342,** 803–804
14. Anderson, P., Caligiuri, M., O'Brien, C., Manley, T., Ritz, J., and Schlossman, S. F. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 2274–2278
15. Letourneur, O., Kennedy, I. C. S., Brini, A. T., Ortaldo, J. R., O'Shea, J. J., and Kinet, J.-P. (1991) *J. Immunol.* **147,** 2652–2656
16. Küster, H., Thompson, H., and Kinet, J.-P. (1990) *J. Biol. Chem.* **265,** 6448–6452
17. Weissman, A. M., Baniyash, M., Hou, D., Samelson, L. E., Burgess, W. H., and Klausner, R. D. (1988) *Science* **239,** 1018–1021
18. Weissman, A. M., Hou, D., Orloff, D. G., Modi, W. S., Seuanez, H., O'Brien, S. J., and Klausner, R. D. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 9709–9713
19. Baniyash, M., Hsu, V. W., Seldin, M. F., and Klausner, R. D. (1989) *J. Biol. Chem.* **264,** 13252–13257
20. Jin, Y. J., Clayton, L. K., Howard, F. D., Koyasu, S., Sieh, M., Steinbrich, R., Tarr, G. E., and Reinherz, E. L. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 3319–3323
21. Huppi, K., Siwarski, D., Mock, B. A., and Kinet, J.-P. (1989) *J. Immunol.* **143,** 3787–3791
22. Letourneur, F., Mattei, M. G., and Malissen, B. (1989) *Immunogenetics* **29,** 265–268
23. Le Coniat, M., Kinet, J.-P., and Berger, R. (1990) *Immunogenetics* **32,** 183–186
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Kishi, K. (1985) *Leuk. Res.* **9,** 381–390

26. Kornbluth, J., Flomenberg, N., and Dupont, B. (1982) *J. Immunol.* **129,** 2831–2839
27. Barsumian, E. L., Isersky, C., Petrino, M. G., and Siraganian, R. P. (1981) *Eur. J. Immunol.* **11,** 317–323
28. Selby, M. J., and Peterlin, B. M. (1990) *Cell* **62,** 769–776
29. MacGregor, G. R., and Caskey, C. T. (1989) *Nucleic Acids Res.* **17,** 2365
30. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–252
31. Gorman,, C. L., Moffat, L., and Howard, H. B. (1982) *Mol. Cell. Biol.* **2,** 1044–1054
32. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489
33. Korner, M., Harel-Bellan, A., Brini, A. T., and Farrar, W. L. (1990) *Biochem. J.* **265,** 547–554
34. Sen, R., and Baltimore, D. (1986) *Cell* **47,** 921–928
35. Angel, P., Imagawa, M., Stein, B., Imbram, R. J., Ramsorf, H. J., Jonat, C., Herrlich, P., and Karin, M. (1987) *Cell* **49,** 729–739
36. Te-Chung, L., and Schwartz, R. J. (1992) *Nucleic Acids Res.* **20,** 140
37. McDonagh, K. T., Lin, H. J., Lowrey, C. H., Bodine, D. M., and Nienhuis, A. W. (1991) *J. Biol. Chem.* **266,** 11965–11974
38. Faisst, S., and Meyer, S. (1992) *Nucleic Acids Res.* **20,** 3–26
39. Hsu, V. W., Klausner, R. D., Fine, J. S., Kruisbeek, A. M., and Baniyash, M. (1992) *New Biol.* **4,** 166–171
40. Klages, S., Mollers, B., and Renkawitz, R. (1992) *Nucleic Acids Res.* **20,** 1925–1932
41. Shen, M. R., Batzer, M. A., and Deininger, P. L. (1991) *J. Mol. Evol.* **33,** 311–320
42. Saffer, J. D., and Thurston S. J. (1989) *Mol. Cell. Biol.* **9,** 355–364
43. Tomilin, N. V., Iguchi-Ariga, S. M. M., and Ariga, H. (1990) *FEBS Lett.* **263,** 69–72