Separate Promoters from Proximal and Medial Control Regions Contribute to the Natural Killer Cell-specific Transcription of the Human FcγRIII-A (CD16-A) Receptor Gene*

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The molecular events governing the differentiation pathway of natural killer (NK) cells are not well understood. The phenotype of mature NK cells is specified by the expression of the low affinity Fc receptor for IgG (human FcγRIII, CD16) encoded by the FcγRIII-A gene. Here we report that the Pprox promoter (−198/−10) of FcγRIII-A stimulated by its own intron enhancer (+10/+712) was only one of the cis-elements that target the expression of a reporter gene in the immature NK cell line, YT. The transcription start sites of the FcγRIII-A a2/3 and a5/6 splice alternatives in NK cells were mapped to the medial (−1817/−850) FcγRIII-A control region. Two promoters, Pmed1 (−942/−850) and Pmed2 (−1376/−1123) reside in this region and controlled for the initiation of these transcript classes encoding the known FcγRIII-A receptor protein. Deletion mapping studies demonstrated that the 93 base pairs (−942/−850) Pmed1 sequence was sufficient to confer cell type-specific expression in YT cells. The 5’ end of Pmed1 (−942 to −921) was required for full promoter function indicating the presence of an important sequence motif recognized by a YT-specific factor. Our data suggest that this motif might be a useful tool for subsequent identification of putative transcription factors uniquely active in YT and NK cells.

Natural killer (NK) lymphocytes are important effector cells in the first line of immunologic defense and play a major role in immunosurveillance. NK cells have the ability to lyse tumor cells and play a crucial role in the control of viral infections. NK cells, like cytolytic T cells (CTL), respond specifically to polymeric determinants of MHC class I molecules. NK cells express receptors that bind to these molecules. However, instead of activating the cytolytic response as in CTLs the recognition of MHC class I turns off the NK cells. Therefore, virus infected and malignant transformed cells are lysed by NK cells as a consequence of loss of MHC class I surface expression (for reviews, see Refs. 1 and 2).

In addition to the allotype MHC class I specific receptors almost all human NK cells express the low-affinity receptor for the Fc portion of IgG, FcγRIII-A (CD16-A). FcγRIII-A belongs to the family of immunoglobulin G receptors (FcyRs) involved in the clearance of immune complexes, phagocytosis of opsonized pathogens, and various forms of antibody-dependent cellular cytotoxicity (3). The FcγRIII-A a chain forms a multimeric transmembrane receptor complex with homo- and heterodimers of the FcγRIγ subunit and/or CD3ζ subunit (4–6). FcγRIII is also highly expressed in PMN but as a single chain receptor attached to the plasma membrane by a glycosylphosphatidylinositol anchor, FcγRIII-B. The transmembrane FcγRIII-A receptor on NK cells mediates antibody-dependent cellular cytotoxicity and all other antibody-dependent responses (7–9). The glycosylphosphatidylinositol-linked FcγRIII-B receptor on PMN is involved in cell activation but its detailed role is less clear (10–12). Other surface molecules like FcγRII and CR3 receptors are likely to be involved in the activation process of PMN after FcγRIII-B cross-linking (13, 14). The molecular basis for the expression of functionally distinct FcγRIII isoforms is given by the presence of the highly homologous FcγRIII-A and FcγRIII-B genes (15). Transfection experiments of reporter gene constructs indicate that differences in the proximal −198/−10 gene promoter regions might be crucial for directing the expression of the FcγRIII-A and B receptors to NK cells and PMN, respectively (16).

The characterization of the molecular events leading to NK cell-specific FcγRIII-A expression is complicated by the finding that transcripts initiating outside the −198/−10 Pprox promoter exist in NK cells (16, 17). Cloning and sequencing of these FcγRIII-A transcripts, designated a2/3 and a5/6, demonstrated that they encode the known FcγRIII-A receptor. The coexpression of FcγRIII-A transcripts with alterations in the extracellular domain were also evident but could not be linked to the distinct a2/3 and a5/6 mRNA start sites. The medial −1817/−850 region of the FcγRIII-A gene containing the a2/3 and a5/6 initiation sites functioned as a transcriptional regulator in the immature NK cell line YT. This control region consisted of the two independent promoters Pmed1 (−942/−850) and Pmed2 (−1376/−1123). The 93-bp −942/−850 Pmed1 promoter was further characterized. It contained a cis-acting DNA element important in conferring optimal and YT cell-specific promoter activity within its first 21 bp. These results suggested that this DNA element might be a useful target for identification of YT and NK cell restricted transcription factors.

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FCYRIII-A Gene Regulation on NK Cells

MATERIALS AND METHODS

Cell Preparations and Culture Conditions—Polymorphonuclear granulocytes (PMN), peripheral blood mononuclear cells (adherent cell fraction, MO), and NK cells were cultured according to standard conditions described elsewhere (9, 16, 18, 19). The γδ T cell (MK1) and cytotoxic T cell (1B3) clones derived from donors with distinct CD16 subsets from sorted fractions were generated by limiting dilution. Clones were plated at 1 cell/well onto a feeder layer with irradiated allogeneic PBL and Epstein-Barr virus-transformed B lymphoblastoid cells (La5s09) (8). Human tumor cell lines HL60 and YT were used for transformation experiments. Cells were cultured in RPMI 1640 containing 10% fetal calf serum and supplements (16). HL60 is a promyelocytic cell line and YT a cell line with NK cell characteristics (20, 21). In some experiments, HL60 cells were induced to express the FCYRIII-B receptor isoform upon culturing in the presence of 1.2% Me2SO (16).

Cloning of FCYRIII-A mRNA Start Sites by RACE PCR—The strategy to obtain cDNA clones for a1, a2/3, and a5/6 related transcription initiation sites was nearly identical to that described recently (16). Starting with 2 μg of poly(A)+ RNA from NK cells, the reverse transcription reaction was performed using 20 pmol of a FCYRIII-A gene-specific primer reverse complementary to EC1 sequences from exon V. The cDNA pools were subsequently tailed with 15 units of TdT (Life Technologies, Inc.) in the presence of 0.1 mm dATP for 10 min at 37 °C. After purification of the reaction mixture, one-fifth was used for PCR amplification with 10 pmol of oligo(dT)25-adaptor, 25 pmol of adaptor, and 25 pmol of a second internal EC1 primer in a total volume of 100 μl. 2 units of Taq DNA polymerase (Promega) was added and the mixture was annealed at 56 °C for 2 min. The tailed cDNA was extended at 72 °C for 30 min. PCR conditions were as described (16). Purified RACE PCR products were digested with SfiI and BgII and cloned into SfiI/BamHI-digested pUC18, as outlined in Fig. 2. Miniprep plasmid DNA was sequenced using the 32P-labeled oligonucleotide. The extension products were annealed to this 32P-end-labeled oligonucleotide. The extension products were analyzed using a 4% agarose gel and visualized by ethidium bromide staining.

RT-PCR Analysis of FCYRIII-A Transcript Classes—Quantitative RT-PCR analysis of FCYRIII-A mRNA start sites occurring outside the Pprox region exist in NK cells. From a total analysis of 11 cloned RACE PCR products 3 cDNA clones assigned as a2/3 were identified to initiate at two sites from 865 to 849 in a separate exon spliced from position 795 to 44 (a2) or to 62 (a3) (16). We now extended the RACE PCR analysis and isolated 17 additional cDNA clones containing the 5′ ends of FCYRIII-A transcripts from NK cells. 7 clones contained the 5′-UTR of transcript class FCYRIII-A a1 (start sites at 20, 27, and 33) and 7 other clones contained a2 but not a3 starting at 865, 871, 877, 881, and 891 (summarized in Figs. 1 and 2). The remaining 3 cDNA clones contained sequences from a further upstream region of the FCYRIII-A gene and represented novel transcript classes assigned as a5/6 (Fig. 2). FCYRIII-Aa5 initiate at positions 1278 and 1254, whereas FCYRIII-Aa6 initiated at 1273. These 5′-untranslated ends of the a5/6 transcripts were encoded by exon 1 ending at position 900 (a5) or alternatively at 946 (a6) and spliced to 44 now recognized as the 5′-border of exon III. This splice site at 44 was used by a2 as well as a5/6 transcripts. That position is the strongest protected fragment in RACE protection experiments from FCYRIII-A expressing NK cells but not from FCYRIII-B expressing PMN (16).

Next, we performed primer extension and RNase protection to determine the a2/3 start sites more precisely. In the RACE protection experiments the FCYRIII-A specific riboprobe ranging from 942 to 707 covering the 3′-border of exon II at 795 from the ATG was used, as described under "Materials and Methods." This analysis was done using RNA from various cell types. Multiple bands were observed preferentially in NK cells and 1B3 T cells, in very reduced amounts in culture activated peripheral blood monocytes but not in U937, HL60, and YT cell lines (Fig. 3). As shown on the right side of Fig. 3 one major protected fragment which mapped to position 887, as well as several minor products ranging from 865 to 889 were observed in NK cells. Consistent with these data, primer extension experiments located the a2/3 transcription initiation sites to the same region, with the major site identified at positions 865 and minor sites at 867, 871, 872, 873, 875, 880, and 884.

We also analyzed transcription initiation from the same region of the FCYRIII-B gene using RNA from Me2SO-treated
The Alternative FcγRIII-A Transcripts a2/3 and a5/6 Encode the Same FcγRIII-A Receptor Isoform in NK Cells—The heterogeneous and the level of transcription of the distinct FcγRIII-A as well as FcγRIII-B mRNAs suggested the presence of simultaneously active but separate transcriptional control regions within each FcγRIII-gene in their respective cell types. Alternative FcγRIII promoters could regulate the tissue-specific expression of transcripts encoding for additional FcγRIII-A or FcγRIII-B gene derived receptor-related isoforms within a single cell. As a first step testing this possibility we performed RNase protection experiments with a riboprobe containing a partially overlapping sequence from exons V/VI encoding the two extracellular EC1 and EC2 domains of FcγRIIIA. For the synthesis of the riboprobe a 147-bp portion of the cDNA p6P5 from the SaII site in exon V to the PvuII site in exon VI was subcloned in sense orientation upstream of the phage T7 promoter within the pKS+ plasmid to generate pEC1–2, as described under “Materials and Methods.” Analysis was done by hybridizing the EC1/EC2 spanning riboprobe made from pEC1–2 to various FcγRIII-A positive and negative RNAs. As expected, in the negative HL60, U937, Jurkat, and YT cell lines and with the control yeast tRNA no FcγRIII-A specific fragments protected by the riboprobe were detected. Interestingly, NK cells, 1B3 cytotoxic T cells, MK1 γ/δ T cells, and culture activated monocytes, all of them encoding the FcγRIII-A receptor on the cell surface, demonstrated coexpression of full-length transcripts along with transcripts containing alterations within the EC1/EC2 domains. As shown in Fig. 4, two protected fragments of 73/74 and of 147 nucleotides can be distinguished. The 147-nucleotide fragment matched the used EC1/EC2 overlap. The 73/74-nucleotide fragment was mainly protected from exon V derived EC1 sequences. Using an exon IV/V overlapping riboprobe only one protected fragment was observed (data not shown). These results indicated the effective transcription of normal FcγRIII-A as well as splice alternatives beyond exon V. Whether such splice variants led to the production of FcγRIII-A related receptors remains to be addressed.

To determine the potential correlation between exon V/VI splice variants and separate clusters of transcription initiation we performed RT-PCR analysis using RNA from NK cells with distinct a2/3 or a5/6 specific 5′-UTR primers and a single 3′ end primer complementary to known FcγRIII-A 3′-UTR sequences, as described under “Materials and Methods.” Using this strategy of amplification, a2/3 and a5/6 main products of 928/946 and 1156/1110 bp were generated indicating no gross alteration within exons V and VI encoding for the extracellular domains (Fig. 5). This was also verified by sequence analysis (data not shown).
Identification of different 5'-UTR exons in the FcγRIII-A gene by RACE PCR cloning the RNA initiation sites of transcript classes a1, a2/3, and a5/6. Top, organization of human FcγRIII-A gene. Exons are shown as boxes: open box, coding region; black box, 5'- and 3'-UTR. Exons are indicated by Roman numerals below each box. The most upstream mRNA start sites for each transcript class a1, a2/3, and a5/6 cloned by RACE PCR and/or mapped by primer extension and RNase protection assays (see Fig. 3) are shown as the distance to the ATG codon indicated by arrows. The position of the three separate promoters associated with the initiation of a1, a2/3, or a5/6 are shown above the FcγRIII-A gene. The variant splice sites are indicated by the lines below the FcγRIII-A gene. Middle, outline of the RACE PCR cloning strategy and analysis of 17 independent RACE-PCR derived FcγRIII-A a1, a2/3, and a5/6 cDNA clones. 1 and 2, strand cDNA synthesis were done as described under "Materials and Methods." Amplified RACE PCR products were digested with SalI and BglII, cloned into SalI/BamHI-digested pKS+, and analyzed by sequencing. The positions of the 5' ends from 17 cDNA clones are shown on the left. Intronic sequences not present in the cDNA clones are indicated by dotted lines. Bottom, parts of two sequencing gels containing the different splice sites detected in the RACE PCR products are depicted; at the left, a2 and a3; at the right, a5 and a6. ACCT or TGCA indicate the sequencing ladders. Alternative splicing at −795/-44 and −795/-62 is shown for a2/3 and at −900/-44 and −946/-44 for a5/6 cDNAs.
shown). Therefore, the transcript classes a2/3 and a5/6 encode for the same FcγRIII-A receptor in NK cells as originally demonstrated for a1 (15).

Proximal and Medial FcγRIII-A Control Regions Act as YT Cell-specific Transcriptional Regulators—Differences in the cell type specific activities of the proximal –198/–10 FcγRIII-A and FcγRIII-B Pprox gene promoters were most evident in combination with enhancer elements. Such elements might be provided by intronic sequences from +10 to +712 between exon III and IV or the more upstream regions from −1817 or −1821 to −198 in both genes, as described recently (16, 17). The newly identified FcγRIII-A and -B transcription initiation clusters now suggest that differential promoter activities would not be restricted to the proximal −198/−10 regions but could also be located to the stimulatory −1817/−1821 to −198 upstream regions.

To examine this possibility we first linked the −1817/−850 and −1821/−846 fragments from the FcγRIII-A/B upstream regions covering with their most 3′ ends the a2/3 or b2 mRNA start sites to the promoterless luciferase gene. The reporter plasmids pIII-A(−1817/−850) + (intr.A)Luc and pIII-B(−1821/−846) + (intr.B)Luc which also contain their respective intron enhancers cloned downstream to the luciferase gene were then transfected into HL60 and YT cells. These two cell lines were used in all our functional studies. Although presenting a more immature phenotype they can serve as model systems for PMN and NK cells, as described earlier (16). As shown in Fig. 6, the −1817/−850 region of FcγRIII-A produced a strong luciferase activity in the immature NK cell line YT. The amount of activity observed in HL60 cells was strongly reduced and a weak expression was seen only in the presence of the intron enhancer. Compared with the complete −1817/−10 and the proximal −198/−10 regions the −1817/−850 sequence caused a significant higher promoter activity but a similar cell type specificity with preferential expression in YT cells. Such promoter activities were not detected in transfected Jurkat T cells and myeloid U937 cells (data not shown). From these data we conclude that in addition to the −198/−10 FcγRIII-A Pprox promoter the −1817/−850 upstream region acts as a YT specific transcriptional regulator. This region will be referred to as the medial control region of the FcγRIII-A gene.

Surprisingly, the same −1821/−846 medial region from the FcγRIIIIB gene did not produce significant luciferase activity in HL60 cells even in the presence of its endogenous intron enhancer. Very low activity could be detected in YT cells. As shown in Fig. 6, this was in sharp contrast to the results when using the complete −1821/−10 and the proximal −198/−10 FcγRIII-B regions. Reporter plasmids containing these latter sequences produced very strong promoter activities specific for HL60 cells. Our data indicate that HL60 cells lack some factors necessary for proper function of the medial but not the proximal FcγRIII-B promoters. Based on these observations we focused in our subsequent analysis mainly on the FcγRIII-A medial control region.

The FcγRIII-A Medial Control Region Consists of the Two Separate Promoters Pmed1 and Pmed2—After establishing the contribution of the FcγRIII-A medial control region in YT cell-specific expression we addressed the question whether the cis-acting sequences from this region were sufficient and responsible for constitutive promoter activity and cell type specificity. Two series of 5′ and 3′ deletion mutants were generated and cloned upstream to the luciferase gene into reporter plasmids lacking the endogenous intron enhancer and tested by transfection into YT cells. This approach provided evidence that the FcγRIII-A medial control region consists of two separate promoters, termed Pmed1 and Pmed2. These two promoter activities appeared to be YT cell specific. All of the deletion mutants were almost negative for luciferase expression when transfected into HL60 cells (data not shown).

As shown in Fig. 7, different FcγRIII-A 5′ deletion mutants originating at position −1817 produced variable amounts of luciferase activities in YT cells suggesting the presence of compensatory enhancer, repressor as well as promoter elements. The original reporter plasmid pIII-A(−1817/−850)Luc produced luciferase activities in the range of 15 to 23 × 103 relative light units (RLU) which were set as 100% relative activity. As an internal standard we used the pTK luciferase control plasmid. Deletion of the most upstream region from −1817 to −1579 drastically reduced activity indicating the presence of a stimulatory element. Further deletion to position −1376

![Fig. 3. Mapping the FcγRIII-Aa2/3 mRNA start sites by RNase protection and primer extension.](image)
Most importantly, these data also suggested that additional Pmed1 sequences reduced the relative activity of the pIII-A element. The importance of the 21-bp end deletion mutants. The deletion of the first 70 bp from the Pmed1 promoter within the pIII-A region for YT specific activity of the Pmed1 promoter account for this residual luciferase expression. To define the region responsible for this remaining promoter activity we generated more extensive 3’ end deletion mutants as well as constructs containing nonoverlapping fragments. As shown in Fig. 7, all the constructs containing the −1376 to −1123 sequence were able to confer activity to the luciferase reporter gene. In the absence of the upstream repressing element of the −1579 to −1376 region the pIII-A/−1376/−1123 reporter construct produced the highest amounts of luciferase activity comparable to what has been observed with the Pmed1 promoter. This region also contains the 5’ ends of the cloned a5/6 RACE PCR products at −1278, −1270, and −1254 and will be referred to as the FcγRIII-A Pmed2 promoter.

To examine whether nucleotide differences were responsible for repressed FcγRIII-B medial promoter activity, some of the FcγRIII-A deletion mutants were compared with their corresponding FcγRIII-B sequences in the functional studies. To test the contribution of each Pmed1 and Pmed2 region, analysis were also done using A/B hybrid constructs. All constructs lacked the intron structures to avoid possible neutralizing effects due to promoter/enhancer competition. As shown in Fig. 8, the complete −1821/−846 medial control region of the FcγRIII-B gene was inactive in its respective HL60 cells and produced some residual activity about 8-fold lower compared to the FcγRIII-A derived sequences in YT cells. Slightly higher levels of luciferase expression were produced by the pIII-B/−947/−846/Luc plasmid, 4-fold lower than observed for the corresponding FcγRIII-A Pmed1 promoter region. The deletion of the −947/−846 region in the pIII-B/−1821/−947/Luc construct resulted in a complete loss of promoter activity, whereas the pIII-A/−1817/−942/Luc containing the FcγRIII-A Pmed2 promoter was still active (data not shown). Similar results were obtained using Pmed1/Pmed2 hybrid constructs from both genes (Fig. 8). Therefore, the residual FcγRIII-B activity resides within the Pmed1 but not the Pmed2 region. Total repression or strong reduction in the FcγRIII-B Pmed2 and Pmed1 activities in YT cells appeared to be mediated by differences in the nucleotide sequence. Comparison of the −1380/−1127 FcγRIII-B and −1376/−1123 FcγRIII-A Pmed2 sequences revealed a single nucleotide exchange of A to G at position −1341 in FcγRIII-B creating a Sp1 consensus site in case of the FcγRIII-A gene (boxed in Fig. 1). As outlined in Fig. 9, the sequence of the FcγRIII-A Pmed1 promoter differed through a truncation of the 3’-UTR region which is three times repeated in the respective FcγRIII-B region. For both genes the main a2/3 or b2 mRNA start sites were mapped.
to positions −887 and −865 in NK cells or −875 in PMN directly downstream to this motif (Fig. 3).

**DISCUSSION**

The human Fc receptors with low affinity for IgG (FcγRIII, CD16) are encoded by two genes (III-A and III-B) resulting in differential tissue-specific expression of alternative transmembrane or glycosylphosphatidylinositol-anchored isoforms in NK cells and PMN, respectively. Sequence conservation of about 97% identity have been described between both coding (15) and flanking (16) regionsofeachgene. Reconstitution studies of the distinct FcγRIII cell type specificities in transgenic mice have indicated that the cis-elements sufficient for NK or PMN restriction might locate to the same 5.8-kilobase fragment containing about 4.5-kilobasepairsofthe5′-flankingsequenceandthefirstintronineachgene(24).

In vitro transfection analyses have demonstrated that the first 0.2-kilobase (−198/−10) of 5′-flanking FcγRIII sequences (16) enhanced by their first introns contribute in directing the expression of a reporter gene to their respective YT (NK-like) or HL60 (PMN-like) cell types (17). The data presented here define transcription initiation upstream of the −198/−10 sequences from both genes, suggesting additional control through alternative promoters. In the case of FcγRIII-B the respective upstream sequences are rather inactive, most likely due to the premature phenotype of HL60 cells used as PMN substitutes (Fig. 6). Our deletion studies indicate that the YT/NK cell-specific expression of FcγRIII-A is dependent on a complex arrangement of transcriptional regulatory regions including a putative enhancer from −1817 to −1579, a suppressor from −1579 to −1376 as well as the two adjacent promoter elements Pmed2 from −1376 to −1123 and Pmed1 from −942 to −850 (Fig. 7).

The engagement of transcription initiation factors by both Pmed1 and Pmed2 regions is supported by the finding that the mRNA start sites of transcript classes a2/3 and a5/6 mapped to both elements in mature NK cells. In all functional studies both Pmed1 and Pmed2 promoters are much more active than the recently characterized (−198/−10) Pprox promoter in YT cells. This is in accordance with RNase protection experiments which identifies the −44 splice site present in a2 and a5/6 as the prominent protected fragment (16). An important question addressed in these studies is whether the distinct transcript classes a2/3 and a5/6 compared to a1 are controlled by alternative promoters and are involved in the tissue-specific expression of more than a single FcγRIII-A receptor isoform in NK cells. The coexpression of mRNAs with or without alterations in exons V/VI encoding the extracellular EC1/EC2 domains (Fig. 4) strongly inferred that alternative splicing participate in FcγRIII-A transcript heterogeneity. On the other hand, RT-PCR amplification with NK cell-derived mRNA yields a pattern of a1, a2/3, and a5/6 products indicative for a single FcγRIII-A receptor (Fig. 5 and data not shown). In addition, characterization of nine cDNA clones isolated from two independent libraries constructed to size-fractionated lymphokine-activated killer or NK cell mRNA identified two clones containing exon II.
sequences specific for a2 which are otherwise identical to the FcγRIII-A coding sequence (data not shown). We propose that evolvement of the separate Pprox, Pmed1, and Pmed2 promoters does not necessarily correlate with the events of alternative splicing within the extracellular domain of the FcγRIII-A receptor. Whether other transcript classes like FcγRIII-Aa4 (16) express for modified FcγRIII-A products remains to be addressed. Based on earlier experiments it is most likely that a4 contains additional 5′ end sequences distinct from a1, a2/3, and a5/6, suggesting that the FcγRIII-Aa4 initiate by a more upstream transcriptional regulator different from Pmed1 and Pmed2 (16, 17).

Results from our functional studies showed high promoter activity within the 92-bp segment (residues −942 to −850) of the FcγRIII-A gene, termed the Pmed1 promoter. Comparison with other parts of the III-A and III-B genes in YT and HL60 cells revealed that differential cell type specificities are due to this region (Fig. 8). An 8-bp repeat motif differed between Pmed1 and the relative FcγRIII-B gene region. This motif, GGAGCCCT, is repeated three times in FcγRIII-B but affected in FcγRIII-A Pmed1 by a C to T exchange in the second repeat and absent in the third repeat (Fig. 9). In YT cells the truncated version present in Pmed1 shows strong improved activity over the FcγRIII-B sequence. Attempts to identify YT-specific DNA-binding proteins in gel retardation assays recognizing this sequence difference were not successful. Therefore, the different organization of the repeat motif might influence binding affinity or oligomerization of a transcription factor rather than destroying binding capability. Possibly, a functional cooperation between the distinct repeat motifs and a further upstream element common to both genes could render only the FcγRIII-A Pmed1 to be specifically active in YT cells. The 21-bp sequence from residue −942 to −921 in the 5′ end of the Pmed1 is required for full promoter function. Deleting this region resulted in almost inactive Pmed1 activity. It is reasonable to assume a cis-element within this region to cooperate with the repeat motif. Sequence comparison revealed a consensus site for Ets proteins, GGAA/T, within this 21-bp segment. Several Ets family members have been shown to be involved in the differential expression of T-cell specific genes, such as the TCR, interleukin-2, and perforin (25–27). The 15-bp NKE motif, CCCACTTCCTGGCCA, bearing the core Ets site is nearly identical to the mPfp CTL-specific element (residues −508 to −494) (Fig. 9). The trans-acting factor NF-P2 exclusively expressed in cytolytic lymphocytes and specifically modulated...
upon activation has been identified to interact with the mPfp 15-mer sequence (24). The coexpression of perforin and FcγRIII-A in some subsets of CTLs, γδ T cells as well as NK cells, suggests that NF-P2 or related proteins may contribute to the FcγRIII-A Pmed1 specificity.

The molecular basis for NK and YT cell-specific transcription is completely unknown. Our data describe for the first time some of the relevant FcγRIII-A cis-acting gene elements includ-
ing Pmed1 and Pmed2. A YT cell-specific sequence motif (NKE) located within the first 21 bp in the Pmed1 promoter element. We propose that a functional cooperation between the NKE and the so-called repeat motif contributes to the YT-specificity of the FcγRIII-A Pmed1 promoter. The identification and characterization of NK cell-specific DNA elements will allow targeting gene expression to NK cells clarifying the role of these important effector cells in the first line of immunologic defense. In addition, NK and YT cell-specific sequence motifs can be used to isolate transcription factors uniquely active in these cell types. Finally, elucidation of the factors involved in the expression of NK cell-specific genes as FcγRIII-A will give insight into the control of NK cell differentiation and development.

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