Human Tumor Necrosis Factor Receptor p75/80 (CD120b)
Gene Structure and Promoter Characterization*

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Sybil M. Santee and Laurie B. Owen-Schaub
From the Department of Immunology, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030

Tumor necrosis factor receptor p75 (TNF-R p75) is a 75-kDa type I transmembrane protein expressed predominantly on cells of hematopoietic lineage. TNF-R p75 belongs to the TNF receptor superfamily characterized by cysteine-rich extracellular regions composed of three to six disulfide-linked domains. In the present report we have characterized, for the first time, the complete gene structure for human TNF-R p75, which spans approximately 43 kbp. The gene consists of 10 exons (ranging from 34 base pairs to 2.5 kilobase pairs) and nine introns (343 base pairs to 19 kilobase pairs). Consensus elements for transcription factors involved in T cell development and activation were noted in the 5′-flanking region including T cell factor-1, Ikaros, AP-1, CK-2, interferon receptor E (IL-6RE), ISRE, GAS, NF-κB, and Sp1. The unusual (GATA), and (GAA)(GGA) repeats found within intron 1 may prove useful for further genome analysis within the 1p36 chromosomal locus. Characterization of the human TNF-R p75 gene structure will permit further assessment of its involvement in normal hematopoietic cell development and function, autoimmune disease, and nonrandom translocations in hematopoietic malignancies.

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†To whom correspondence should be addressed: M. D. Anderson Cancer Center, Dept. of Immunology, Box 178, 1515 Holcombe Blvd., Houston, TX 77030. Tel.: 713-792-8735; Fax: 713-745-0846; E-mail: laurieowen-schaub@isqm.mda.uth.tmc.edu.

‡The abbreviations used are: TNF, tumor necrosis factor; TNF-R, TNF receptor; LT, lymphotixin-α (LT-α); IL, interleukin; CMV, cytomegalovirus; GAS, γ-interferon activation sequence; PCR, polymerase chain reaction; μF, microfarads; TCF, T cell factor; CMV, cytomegalovirus; rev, reverse orientation; ISRE, interferon-stimulated response element.

Our data demonstrate that the TNF-R p75 locus is contained within 10 exons spanning approximately 43 kbp. These studies provide the first report of the complete gene structure for...
human TNF-R p75. Several consensus elements for transcription factors involved in lymphoid development and activation were noted in the 5'-flanking region including T cell factor-1 (TCF-1), Ikaros, AP-1, CK-2, IL-6RE, GAS, NF-kB, and Sp1. This region was verified to contain promoter activity by transient transfection into several cell lines.

MATERIALS AND METHODS

Identification of Human TNF-R p75 cDNA Clones—A cDNA library was constructed in Xgt11 using total RNA obtained from activated human lymphocytes (cultured in IL-2 and anti-CD3 for 8 days) (Promega Corp.). A murine TNF-R p80 cDNA probe (the generous gift of Genentech, Inc., South San Francisco, CA) was used to screen approximately 750,000 plaque-forming units in duplicate. In brief, hybridization was performed in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 4%–10% polyethylene glycol (MW > 40,000) at 55°C in 2 × SSC (23). Positive clones were purified to donality, and cDNA inserts (EcoR1-Ncol) were subcloned into the plasmid pGEM11zf(+) (Promega) for restriction mapping and sequencing using Sp6 and T7 primers (Sequenase V2; U.S. Biochemical Corp.). The human TNF-R p75 cDNA was found to contain nucleotides 408 to 2364 bp.

Identification of TNF-R p75 Genomic Clones—A cosmid library in pCOS8 consisting of human placental genomic DNA (kindly provided by Dr. David Lawlor, UTMDACC) was screened according to established protocols (25). In brief, the library was plated (5 × 10⁶ clones) on sterile nitrocellulose filters, transferred and screened in duplicate on nylon membranes. Fragments were subcloned into pGEM11zf(+) and verified by restriction and sequence analyses to contain exon 1 and intron 1. The 5.0-kb fragment (putative promoter) was subcloned into the NheI site within the multiple cloning region of the promoterless enhancerless luciferase vector pGL2-basic (Promega) and verified by restriction and sequence analysis to contain the region immediately upstream of exon 1. The 5'-NheI site occurs within the multiple cloning region of the plasmid and is not encoded within the genome. Subsequently, the 7.0-kb NheI fragment was further subcloned into the pGL2-basic vector using an internal HindIII site and a HindIII fragment contributed by the pGL2-basic polylinker to provide a 1.8-kb HindIII fragment immediately upstream of the ATG. Clones for both the 7.0-kb NheI and 1.8-kb HindIII pGL2-basic constructs were obtained in both orientations corresponding to transcript polarity. The 1.8-kb HindIII fragment in pGL2-basic was sequenced in one direction after exonucleolytic digestion (Erase-A-Base System, Promega). Sequence data was examined using MacVector Sequence Analysis software (IBI, New Haven, CT) for promoter and enhancer consensus sequences.

Cell Lines—K562 (erythroleukemia), U-937 (promyelocytic leukemia), Jurkat (T cell leukemia), MOLT4 (T cell leukemia), and HSB-2 (T cell leukemia) were obtained from American Type Culture Collection (Rockville, MD). CEM (T cell leukemia) was kindly provided by Dr. David McConkey, UTMADCC. Jurkat, K562, CEM, MOLT4, and U-937 were grown in RPMI 1640 supplemented with 2 mM glutamine, 10% FCS, and 10% fetal bovine serum and maintained in a humidified incubator. HSB-2 was cultured in Iscove’s modification of Dulbecco’s media supplemented with 10% fetal bovine serum, 2 mM glutamine, and 10% FCS.

Transfections and Luciferase Assay—Supercoiled plasmid DNA was transfected into cell lines using the following protocols. K562 cells were transfected with 20 μg of DNA by electroporation using the BTX Electroporation Cell Manipulator 600 (BTX, Inc., San Diego, CA) in serum-free RPMI with the following parameters: 600 μF, 13-ohm resistance, 360 V. 3.6 × 10⁶ cells were transfected with 50 μg of plasmid DNA by electroporation using the BTX Electroporation Cell Manipulator 600 with the following parameters: 2850 μF, 129-ohm resistance, 110 V. U-937 cells, Jurkat, and HSB-2 were transfected by electroporation using a Bio-Rad electroporator. U-937 cells were transfected with 20 μg of plasmid DNA, described (26). 3 × 10⁶ T cell leukemia cells in 0.3 ml of RPMI supplemented with 20% fetal bovine serum were transfected with 10 μg of plasmid DNA by electroporation with the following parameters: 270 V, 900 μF, 6 × 10⁶ HSB-2 cells in 0.3 ml RPMI supplemented with 10% fetal bovine serum were transfected with 15 μg of plasmid DNA by electroporation with the following parameters: 300V, 900 μF.

After a 48-h incubation (8 h for U-937 transfectants), cell extracts were prepared with cell culture lysis reagent (Promega) using approximately 1 × 10⁶ cells/200–400 μl of lysis reagent. 20 μl of extract was assayed for luciferase activity by the addition of 100 μl of luciferase reagent (Promega). The signal was integrated over 20 s using a Turner Designs luminometer, TD-20e (Promega). Luciferase activity is expressed as relative luciferase units/mg of protein. Protein concentration was measured using the Bio-Rad protein assay reagent (Bio-Rad) ac-
According to the manufacturer's recommendations, fold activity is expressed as the ratio of the test construct compared with a promoterless control, pGL2-basic (Promega). The pGL2-control vector (Promega) and the CMV-luc vector containing the luciferase gene under control of an SV40 promoter/enhancer and the CMV promoter, respectively, were used as positive controls. CMV-luc was constructed by replacing the SV40 promoter of the pGL2-control vector with the CMV promoter.

RESULTS

Identification of TNF-R p75 Genomic Clones—Two human genomic libraries constructed with placental genomic DNA were screened to obtain clones containing the gene for human TNF-R p75. Initially, \(5 \times 10^5\) cosmid clones in the pCOS8 vector were screened with a cDNA probe containing nucleotides 408–2364 of the published sequence (24). Several positive cosmid clones were obtained; clone 10.1.2.1 (also positive using a probe containing nucleotides 408–643) was further characterized by subcloning SacI restriction enzyme fragments. The SacI fragments were further analyzed by Southern analysis, restriction enzyme mapping and DNA sequencing. Because the 5'-untranslated exon 1 region was not represented among these fragments, a 5'-most fragment (2.2-kb NotI-NsiI of 10.1.2.1) was used to screen additional cosmids to obtain overlapping clones. The resultant cosmid clone, 7.7.1.1 (Fig. 1), contained nucleotides in the 5'-untranslated exon 1 region in addition to those contained in 10.1.2.1, with minimal sequence (0.5 kb) upstream of exon 1. After failing to obtain a significant region upstream of exon 1 in three distinct cosmids, we screened a human genomic library in EMBL3 using the 5'-end (4.3-kb EcoRI-SphI fragment) of cosmid 7.7.1.1 and identified a λ phage clone 17.1.1.1 (Fig. 1) containing an additional 6.6 kbp upstream of exon 1. Together, the two cosmids clones and one λ phage clone represent the entire coding sequence for human TNF-R p75 (24) (Figs. 1 and 2).

Characterization of TNF-R p75 Genomic Clones—Alignment and gene sequence of the two cosmid clones 10.1.2.1 and 7.7.1.1 as well as the λ phage clone 17.1.1.1 relative to the cDNA is shown in Figs. 1 and 2. Exon-intron boundaries were shown to obey the GT-AG rule for splice junction sequences (Table I) (27). Cosmid 10.1.2.1 contained exons 2–10 (nucleotides 175–3684) (Fig. 1). The overlapping cosmid, 7.7.1.1, contained additional sequence 5'-untranslated and exon 1 sequences. In addition to traditional subcloning techniques, exonuclease III digestion was employed to facilitate sequencing; therefore, partial, if not complete, sequence was obtained for most of the gene. Surprisingly, five SacI restriction enzyme fragments (cosmid 10.1.2.1) constituted an approximately 12-kb EcoRI fragment partially spanning intron 1. Although the fragment linking the SacI fragment containing exon 2 to the 12-kb EcoRI fragment was not obtained, the size of intron 1 was estimated to be approximately 19 kb based on the total cosmid size of 39 kb and the upper limit of insert sizes within the cloning vector. Since three distinct cosmids and four distinct λ phage clones obtained from two independent libraries displayed similar restriction maps by agarose gel electrophoresis and Southern analysis, this unusually large intron is un-
FIG. 2. DNA sequence of human TNF-R p75. A, complete DNA sequence and corresponding amino acid sequence of TNF-R p75 is shown from the ATG to the stop codon. Exons and introns are denoted by uppercase and lowercase letters, respectively. Intron sizes are indicated within the dashes and the transmembrane domain is underlined.

B, DNA sequence of the 5′-regulatory region is shown. Numbering is relative to the ATG.
likely to be the result of a cloning artifact. Exon 5 and part of exon 9 were not represented in the initial SacI subclones characterized and were obtained by PCR amplification of human genomic DNA using intron- and exon-specific primers (see "Materials and Methods").

Intron 1 Contains Two Repetitive Elements—Two repetitive elements were evident upon sequence analysis of intron 1. The first is a (GATA)n sequence spanning 614 bp sharing 74.4% identity over a 511-bp overlap with murine low affinity IgE receptor (28) (Fig. 3A). These (GATA)n repeats show extensive polymorphism and have been implicated in recombination events (29). The second repetitive element is a (GAA)(GGA) repeat spanning 154 bp (Fig. 3B) sharing greater than 80% identity to murine and human simple sequence repeats (30). This repeat is similar to sequences that have been shown to form H-DNA (triplex DNA) (31).

Polymorphisms within the Human TNF-R p75 Gene—A partial human cDNA clone for TNF-R p75 was obtained from a λgt11 IL-2/anti-CD3-stimulated lymphocyte cDNA library (27). Several simple polymorphisms resulting in three nonconservative amino acid changes had been noted upon comparison of the published TNF-R p75/80 cDNA clones (32). We also detected three additional polymorphisms within the nontranslated region of exon 10 (Table I). We next compared the TNF-R p75/80 gene nucleotide sequence to the cDNA sequences to determine the presence of these simple polymorphisms. Our cDNA clone was identical to that of Smith et al. (24) and Dembic et al. (33) and differed from that of Kohno et al. (34) and Heller et al. (35) at one and three sites, respectively. The cosmid 10.1.2.1, which contained exons 2–10 agreed with the sequence of Smith et al. (24) at all three sites of nonconserved amino acid changes; however, 10.1.2.1 differed at all three polymorphisms within the noncoding region of exon 10. The significance of these changes, if any, is unclear.

Identification of a Putative 5'-Promoter Region—As previously discussed, a phage clone designated 17.1.1.1 (Fig. 1), containing exon 1, a portion of intron 1 and approximately 7.0 kb 5' of the ATG start codon, was identified. Complete sequence analysis of the 1.8-kb HindIII fragment of 17.1.1.1 was obtained by exonuclease III digestion and primer walking. Two nucleotide changes from the published human TNF-R p75 promoter were noted at positions –1413 (A → C) and –1120 (G → C) (36). The change at –1120 creates an Apal restriction enzyme site. A computer search of this region demonstrated consensus sequences for previously described transcription regulatory elements: TATA boxes at –913, –939, and –955 (37);

TABLE I
Exon-intron organization of the human TNF-R p75 gene
Exon sequences are in capital letters, and intron sequences are in lowercase letters. Position numbers refer to cDNA as numbered in Smith et al. (24). Consensus sequences are given in the headings.

| Exon no. | Exon size | Position | 5' splice donor (A/C)AG | GT(A/G)AGT | Intron size | 3' splice acceptor (Y)6C | AT(G/T) |
|----------|-----------|----------|------------------------|------------|------------|------------------------|---------|
| 1        | 174       | 1–174    | CAG                     | gtgggtga    | <19,000     | tctctctctcag           | GTG     |
| 2        | 99        | 175–274  | CCG G                   | gtgagggc    | >205       | tctctctctcag           | GC      |
| 3        | 121       | 275–396  | CCA G                   | gtacgggg    | 673        | tctctctctcag           | AC      |
| 4        | 149       | 397–546  | CAG                     | gtgagtag    | 510        | tctctctctcag           | ACT     |
| 5        | 102       | 547–649  | AT                      | gtgagtag    | 343        | tctctctctcag           | C       |
| 6        | 235       | 650–885  | GTT G                   | gtaagtgc    | 912        | tctctctctcag           | GA      |
| 7        | 68        | 886–954  | AAA A                   | gtaagagt    | 549        | tctctctctcag           | AG      |
| 8        | 34        | 955–989  | GTG                     | gtgagtag    | 6817       | tctctctctcag           | CT      |
| 9        | 203       | 990–1193 | TCA G                   | gtaagagg    | 4995       | tctctctctcag           | AT      |
| 10       | 2476      | 1194–3670|                         |            |            |                        |         |

Polymorphisms are indicated by a double underline at positions –1413 and –1120. Restriction enzyme sites are indicated above the line. Consensus sequences for transcription factors are underlined and indicated below the line. This sequence has been submitted to the GenBank database under the accession number U53483.
two GC boxes at –55 (rev) and –159 (37); two binding sites for TCF-1 at –843 and –1084 (rev) (38); two GAS sites at –364 and –1578 (39); a CAMP-responsive element at –763 (38); an AP-1 site at –1604 (38); two binding sites for NF-κB at –1517 and –1890 (rev) (38); a binding site for Ikaros transcription factor at –1821 (38); and a binding site for interferon regulatory factor-1 (IRF-1) at –113 (40). These results suggested that phase 17.1.1.1 contained the 5’-regulatory region of human TNF-R p75.

In addition to the regulatory consensus elements, the 5’ promoter region was noted to contain a high percentage of GC (approximately 80% within the first 500 bp of the ATG) and a high frequency of CpG dinucleotides (frequency of 0.072). For the sake of comparison, the CpG frequency can be compared with an average CpG frequency within the human genome of 0.008. This unusually high GC content is also observed within the promoters of Fas, TNF-R p60, and CD40.

Transcriptional Activity of the Putative Promoter Region—Upon isolation of the 5’-flanking region of TNF-R p75, transcriptional activity of this region was determined by transient transfection of luciferase reporter constructs. To test for promoter activity, two luciferase reporter plasmids were constructed in the pGL2-basic vector. The first construct contained the 7.0-kb NheI fragment (pGL2–7.0-kb NheI) and the second contained the 1.8-kb HindIII fragment (pGL2–1.8-kb HindIII) (see “Materials and Methods” and Fig. 4). The pGL2–1.8-kb HindIII construct was chosen for further analysis. Control vectors used included pGL2-control vector (luciferase gene under the control of the SV40 promoter/enhancer) and CMV-luc vector (luciferase gene under the control of the CMV promoter/enhancer). Cell lines used for analysis included J urkat, CEM, MOLT-4, and HSB-2 (T cell–derived leukemias), U-937 (promyelocytic cell line), and K562 (erythroleukemia cell line). J urkat, CEM, and MOLT-4 are negative for TNF-R p75, while U-937, HSB-2, and K562 express TNF-R p75 as measured by flow cytometry (Fig. 5). -Fold luciferase activity (expressed as relative luciferase units/ng of protein compared with the promoterless vector pGL2-basic) was shown to vary among the cell lines from approximately 13-fold in the case of K562 to 100-fold in the case of U-937 (Figs. 6 and 7). In cell lines negative for TNF-R p75 expression (MOLT4, CEM, J urkat) the 1.8-kb HindIII construct was only minimally active. In all cell lines tested, the 1.8-kb HindIII construct in the opposite orientation relative to the luciferase gene was negative for promoter activity (data not shown). Taken together, these results demonstrate that the 1.8-kb region upstream of the ATG contains a functional promoter.

**DISCUSSION**

We report here the complete gene structure for human TNF-R p75. The genetic characterization of TNF-R p75 has lagged behind that of other TNF-R superfamily members. The gene structure has been reported for TNF-R p55 (10 exons) (41), CD27 (6 exons) (42), CD40 (9 exons) (43), Fas antigen (9 exons) (44), 4-1BB (10 exons) (45), and OX40 (7 exons) (46). However, partial characterization of the TNF-R p75 promoter region and a portion of intron 1 and exon 2 has been recently reported (36). Our studies confirm and extend the previous findings and demonstrate that the entire coding sequence for TNF-R p75 is contained on 10 exons separated by nine introns spanning approximately 43 kb in the genome. The unusually large architecture of the TNF-R p75 gene relative to other family members is attributable to the size of the first and ninth introns, approximately 19 and 6.8 kb, respectively. A comparison of human TNF-R p75 gene structure with that of other family members as described by Birkenland et al. (46) demonstrates conservation of a subset of the intron/exon borders, further supporting the hypothesis that this superfamily evolved from a primordial gene containing the cytokine-rich repeating structure with subsequent duplication and divergence by the addition of introns by random integration. Although the cDNA and protein sequences of TNF-R p55 and p75 are no more similar to each other than to the other family members, they do share, along with 4-1BB, a 10-exon/9-intron gene structure.

The promoter region of human TNF-R p75 was analyzed genetically by sequence analysis and functionally by transfection of luciferase-reporter constructs into cell lines. Our sequence data differed from the published sequence at positions –1413 (A → C) and –1120 (G → C) (36). The change at –1120 creates an Apal restriction enzyme site. Sequence analysis within the proximal 1.8 kb of the ATG demonstrated the presence of consensus elements for transcription factors known to be involved in lymphoid activation and/or development. Consensus elements for TATA boxes are located at positions –939, –955 (37). Two GC boxes are located at –155 (rev) and –159 (37). Two binding sites for TCF-1 are located at –843 and –1084 (rev) (38). TCF-1 is a high mobility group protein that is involved in the regulation of CD3ε and T cell receptor β and δ enhancers and is preferentially expressed in T cells. Two GAS

**TABLE I**

| Exon | Smith et al. (24) | Kodino et al. (34) | Heller et al. (35) | Dembic et al. (33) | Santee $^a$ | Genome $^a$ |
|------|-----------------|-------------------|-----------------|------------------|--------|--------|
| 4    | Arg-143         | —                 | Pro             | —                | —      | —      |
| 6    | Met-198         | Arg               | Thr             | —                | —      | —      |
| 9    | Ala-365         | —                 | —               | —                | —      | —      |
| nt 1665 A | —             | —                 | NA              | —                | —      | —      |
| nt 1668 G | T             | NA                | —               | T                | G      | —      |
| nt 2007 T | C             | NA                | —               | T                | T      | —      |

$^a$ Present work.
sites are located at -454 and -1667 (39). GAS-like elements were recently shown to be critical for the regulation of murine IL-2 receptor α-chain expression by IL-2 (47). Since IL-2 also regulates TNF-R p75 expression in T lymphocytes (48), these sites are intriguing candidates for regulation of TNF-R p75. A cAMP-response element is located at -2851 (38). cAMP was shown to be involved in the transcriptional regulation of TNF-R p75 in U-937 cells (49). Similarly, PKC was shown to transcriptionally regulate TNF-R p75 in HL-60 cells (50). In this regard, an AP-1 site is located at -21604 (38) and is likely to be involved in the induction of TNF-R p75 by PKC. Two NF-κB binding sites are located at -1517, -1902, and rev (38). Several cytokines and mitogens such as IL-2 (48), IL-1 (51), TNF (51), and lipopolysaccharide (52) have been shown to regulate TNF-R p75 expression in T cells and macrophages and also to induce NF-κB activation (53). A binding site for the Ikaros transcription factor is located at -1821 (38). The Ikaros transcription factor is involved in development of the lymphoid lineage as evidenced by an absence of T and B lymphocytes in Ikaros knockout mice (54). A binding site for IRF-1 is located at -113 (40). TNF, in addition to inducing NF-κB, has been shown to also activate IRF-1 (55). Lipopolysaccharide-γ has been shown to both induce and repress cell survival and mRNA expression of TNF-R p75 (52, 56) and may exert its effects at the GAS-like or IRF-1 consensus elements.

Since TNF-R p55 and p75 are differentially expressed, we would predict few similarities within their 5′-regulatory regions. TNF-R p55 is constitutively expressed at low levels on most cell types, whereas TNF-R p75 is restricted to cells of the hematopoietic lineage. Additionally, TNF-R p75 is inducible upon activation of T and B lymphocytes. This prediction of few similarities between the two promoters is supported by the TNF-R p55 5′-regulatory region possessing features of a housekeeping promoter and its lack of inducibility (57, 58). Although further analysis of the TNF-R p75 promoter is required, there are several candidate elements, for example the GAS, NF-κB, and AP-1 elements, that may contribute, perhaps in a cooperative fashion, to the cell-type specificity and inducibility of the TNF-R p75 promoter as has been demonstrated for the IL-2 receptor α-chain gene. It is intriguing to note that the TNF-R p75 promoter is more similar to the human Fas/APO-1 promoter with regard to putative regulatory elements. This is not surprising, since both genes are expressed upon activation of T and B lymphocytes (44).

In addition to the consensus sequences found within the 5′-regulatory region of human TNF-R p75, a region of high CpG frequency and GC content was evident within the first 500 bp
of the ATG (Fig. 4). A comparison of the CG content and frequency of CpG islands revealed an interesting characteristic of the TNF-R family analyzed to date. Within the first 500 bp of the ATG, human TNF-R p75 has a CpG frequency of 0.072 (36 sites) and varies between 50 and 85% GC. If this is extended to include exon 1, the frequency increases to 0.096 (48 sites). Human Fas, human CD40, murine TNF-R p55, and murine CD40 demonstrate a CpG frequency of 0.054 (27 sites), 0.05 (25 sites), 0.048 (24 sites), and 0.028 (14 sites), respectively (59). The average CpG frequency of eukaryotic DNA is 0.008 with an average GC content of 40% (60). The lack of a consensus TATA box and high GC content is a hallmark of so-called “housekeeping” genes. Although Fas, CD40, and TNF-R p55 promoters would seem to fall into this category with a lack of a consensus TATA box, high GC content, and multiple transcription initiation sites, human TNF-R p75 does contain several consensus TATA boxes. We were unable, however, to determine whether these TATA boxes were functional, since Northern analysis, RNase protection, and primer extension studies were inconclusive. In this regard, the high GC content within this region likely resulted in the nonspecific interaction of probes and oligonucleotides.

Functional analysis of the putative promoter region was accomplished by transfection of promoter-luciferase constructs into a variety of cell lines. The region 1.8 kb 5′ to the ATG was shown to drive luciferase expression in several cell lines of T, myeloid, and erythroid lineages that express TNF-R p75 with relative luciferase units ranging from 13- to 100-fold. In cell lines negative for TNF-R p75 expression, this construct was only minimally active. Although the transcription initiation site was not determined, the ability of this construct to drive luciferase expression suggests that a functional promoter is located within 1.8 kb of the ATG. The presence of the (GATA)₃ tetrameric repeat and the (GAA)₃ tetrameric repeat within intron 1 may allow for a more refined genome analysis within the 1p36 region, and these may be candidate sites for possible translocations within this region. Tetrameric and trimeric repeats (simple sequence repeat polymorphisms or microsatellites) occur every 300–500 kb throughout the genome (61). The polymorphic nature and increased heterozygosity of these repeats compared with current markers make them ideal candidates for physical and genetic mapping of the human genome and for disease diagnosis (62). Such repetitive elements are thought to contribute to chromosomal translocations and deletions (29) and are possible sites for translocation known to occur within the 1p36 locus (15–19). Additionally, polypurine-polypyrimidine sequences have been shown to form DNA triplexes (H-DNA) (31). The effects of such structures on replication and transcription are yet unclear.

Recently, two simple polymorphisms within the 3′-untranslated region of human TNF-R p75 were identified using the single-strand conformation polymorphism technique (PCRSSCP). These polymorphisms have been subsequently utilized in linkage analysis to confirm the placement of TNF-R p75 at the 1p36.2-1p36.3 locus and proximal to the pronatriodilatin (PND) gene, which had been previously used for restriction fragment length polymorphism linkage analysis (63). In addition to these polymorphisms located within the 3′-untranslated region, three nonconservative amino acid changes were also observed in human TNF-R p75 cDNA clones (32). We, therefore, compared our genomic clone with that of the published cDNA sequences. Our genomic clone was identical to the published cDNA sequence of Smith et al. (24) at the three nonconservative amino acid residues; however, our genomic clone differed from that of Smith et al. at three other noncoding region polymorphisms located within exon 10.

The precise roles of the TNF-Rs in T cell activation, proliferation, and death remain unclear. TNF-R p75 has been postulated to play a passive role in signaling via the TNF-R p55 receptor, the so-called “ligand passing model” (64). Recent data, however, suggests that TNF-R p75 can play an active role in signal transduction, including the induction of proliferation in thymocytes (7) and peripheral T lymphocytes (65), mediation of apoptosis (66), elicitation of cytokine secretion (9), peripheral deletion of activated CD8+ T cells (67), and inhibition of primitive hematopoietic progenitors (10). The disparity in the function of TNF-R p75 in cytotoxicity and activation of hematopoietic cells may partially be explained by the differences in ligand binding between TNF-R-p55 and p75. Recent evidence indicates the transmembrane form of TNF-α is the prime activating ligand of TNF-R p75 and can give qualitatively different results compared with soluble TNF-α (68). Tumor cells that are resistant to soluble TNF-α could be made sensitive by activating TNF-R p75 with the transmembrane form of TNF-α. Thus, TNF-R p75 activation may be more important in localized, inflammatory responses. Along these lines, recent evidence suggests that TNF-R p75 is responsible for peripheral deletion of activated CD8+ T cells, whereas Fas-Fasl is responsible for peripheral deletion of CD4+ T cells (67). However, TNF-R p75-deficient mice produced by homologous recombination failed to show a striking phenotype, demonstrating only a reduction in TNF sensitivity and a decreased necrotic effect of subcutaneously injected TNF (69). Compensation for the loss of TNF-R p75 by other family members may be masking an apparent role of TNF-R p75 in immune function.

Given the apparent inconsistencies in the literature, further characterization of TNF-R p75 at the genetic level will be required to clarify its role in immune function and pathologic states such as autoimmune, HIV infection, and lymphoid malignancies (1). Indeed, genetic abnormalities near the TNF-R p75 locus on human and mouse chromosomes 1 and 4, respectively, have been observed in several pathologic states such as hematopoietic cell malignancies (15), neuroblastoma, glioma, and cervical and ovarian carcinoma (13, 20). With respect to the latter, it is interesting to note that soluble TNF-R p75 present in ovarian ascites has been shown to correlate with disease progression (70). It is tempting to speculate that dysregulated TNF-R p75 expression in such cancer cells may result in the neutralization and clearance of TNF produced locally by activated macrophages and/or lymphocytes and permit escape from immune surveillance, as has been shown for malignant keratinocytes and melanocytes (71).

Determination of the human TNF-R p75 gene structure will allow assessment of its involvement in autoimmune disease and in nonrandom translocations observed in some hematopoietic malignancies. Additionally, cloning of the 5′-flanking regulatory region of human TNF-R p75 will facilitate further analyses of activation stimuli and transcription factors involved in the regulation of hematopoietic cells.

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