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Immunology

Cloning of the cDNA for a hematopoietic cell-specific protein related to CD20 and the β subunit of the high-affinity IgE receptor: Evidence for a family of proteins with four membrane-spanning regions

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ABSTRACT **We report the cloning of the cDNA for a human gene whose mRNA is expressed specifically in hematopoietic cells. A long open reading frame in the 1.7-kb mRNA encodes a 214-aa protein of 25 kDa with four hydrophobic regions consistent with a protein that traverses the membrane four times. To reflect the structure and expression of this gene in diverse hematopoietic lineages of lymphoid and myeloid origin, we named the gene HTm_4. The protein is about 20% homologous to two other "four-transmembrane" proteins; the B-cell-specific antigen CD20 and the β subunit of the high-affinity receptor for IgE, $Fc_εRIβ$. The highest homologies among the three proteins are found in the transmembrane domains, but conserved residues are also recognized in the inter-transmembrane domains and in the N and C termini. Using fluorescence *in situ* hybridization, we localized HTm_4 to human chromosome 11q12–13.1, where the CD20 and $Fc_εRIβ$ genes are also located. Both the murine homologue for CD20, Ly-44, and the murine $Fc_εRIβ$ gene map to the same region in murine chromosome 19. We propose that the HTm_4, CD20, and $Fc_εRIβ$ genes evolved from the same ancestral gene to form a family of four-transmembrane proteins. It is possible that other related members exist. Similar to CD20 and $Fc_εRIβ$, it is likely that HTm_4 has a role in signal transduction and, like $Fc_εRIβ$, might be a subunit associated with receptor complexes.**

The high-affinity receptor for IgE, $Fc_εRI$, is part of a tetrameric receptor complex consisting of an α chain, a β chain, and two γ chains (1). Together, they mediate interaction with IgE-bound antigens leading to such dramatic cellular response as the massive degranulations of mast cells. Thought until recently to be expressed only in mast cells and basophils, $Fc_εRI$ has been shown to be present also in Langerhans cells (2), eosinophils (3), and peripheral monocytes (4). The β subunit, $Fc_εRIβ$, is a four-transmembrane protein with both the N and C termini residing in the cytoplasm (2). The human CD20 antigen (5), as well as its murine equivalent, Ly-44 (6), are expressed only in B cells. There is no evidence that CD20 is associated with any other protein. Functional studies with different CD20 antibodies indicate that CD20 is involved in the regulation of B-cell activation (7). CD20 also contains four transmembrane domains with the N and C ends on the same, cytoplasmic side of the cell membrane. There is an overall amino acid similarity of 16% between CD20 and $Fc_εRIβ$. Furthermore, the murine $Fc_εRIβ$ gene maps to the same region in chromosome 19 as the Ly-44 gene (8). It has therefore been speculated that these genes might have evolved by duplication and that similar four-transmembrane proteins might be found in other receptor complexes (2).

We have undertaken a search for hematopoietic cell-specific genes with the expectation that the identification of such genes should widen the current view of regulatory molecules and specialized mechanisms governing the development and function of cells of the hematopoietic system. We report here the identification of a cDNA for a four-transmembrane protein¶ with significant homology to $Fc_εRIβ$ and CD20. The mRNA is detected in both lymphoid and myeloid hematopoietic lineages but not in a wide range of nonhematopoietic cells. Hence, we call the gene HTm_4. By comparing the predicted protein structures of HTm_4, $Fc_εRIβ$, and CD20, and from the chromosomal localization of the gene, we propose and define the characteristics of what appears to be a family of four-transmembrane proteins. The gene should also be relevant to the study of Fc receptors.

MATERIALS AND METHODS

Cell Lines and Primary Cells. Hematopoietic cell lines used included lymphomyeloid (DU528), erythroleukemic (K562, OCIR), promyelocytic (HL-60), myeloblastic (KG-1), monoblastic (U937), T-cell leukemia/lymphoma (MOLT-4, Ly17, Ly13), and myeloma (OCI-My5) lines. Nonhematopoietic cell lines used included bone marrow stromal (BS-1), hepatoma (Hep G2), melanoma (Hs294), skeletal muscle (HuSk), neuroblastoma (SKNSH), cervical cancer (HeLa), and lung cancer (Calu-1) cells. All cell lines were maintained in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum and 1 mM L-glutamine, except for DU528, for which horse serum replaced fetal bovine serum. Total RNAs from a human mast cell line, HMC-1 (9), and a human factor-dependent megakaryocytic line, MO7e, were kindly provided by Karl Nocka (Cytomed, Cambridge, MA). Normal bone marrow cells were harvested from transfusion filters after bone marrow transplantations. Primary leukemic cells with >90% blasts were harvested from the peripheral blood of a patient with M4 acute myeloid leukemia (AML). Total RNAs of neutrophils and eosinophils from normal individuals and eosinophils from a patient with hypereosinophilic syndrome (HES) were kindly provided by Peter Weller and Kaiser Lim (Harvard Medical School).

Preparation of Probes from Subtractive cDNA Libraries for Differential Screening. The construction of four subtractive cDNA libraries (DU528/BS-1, K562/BS-1, KG-1/BS-1, and

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Abbreviations: AML, acute myeloid leukemia; FISH, fluorescence *in situ* hybridization; HES, hypereosinophilic syndrome; PMA, phorbol 12-myristate 13-acetate; RT, reverse transcription.

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¶The sequence presented in this paper has been deposited in the GenBank data base (accession no. L35848).

BS-1/BS-1) from three human hematopoietic cell lines (DU528, K562, and KG-1) and one nonhematopoietic human cell line (BS-1), using the pT3T719U multiphagemid vector (Pharmacia), was described previously (10). cDNA inserts released from two of the hematopoietic (DU528/BS-1 and KG-1/BS-1) and the nonhematopoietic (BS-1/BS-1) subtractive libraries were purified, labeled with ^{32}P, and used as probes to screen the K562/BS-1 library (11).

Induction of Cell Line U937. The U937 cell line was grown to 5×10^5 cells per ml and differentiation was induced with 50 nM phorbol 12-myristate 13-acetate (PMA; Sigma).

Chromosomal Localization of the HTm_4 Gene. The HTm_4 gene was localized by fluorescence *in situ* hybridization (FISH) (12). Human metaphases were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. The HTm_4 cDNA was labeled by nick-translation with biotin-11-UTP (Enzo Diagnostics). The biotin-labeled probe was hybridized to metaphase cells and detected with fluorescein-conjugated avidin (Vector Laboratories). Slides were examined by two observers without knowledge of the probe used.

Reverse Transcription (RT)–PCR. RT was carried out (13) with RNAs from cell lines or cells enriched for various cell types to obtain first-strand cDNAs. The cDNAs were subjected to PCR amplification (14) using primers spanning nt 721–1087 of HTm_4 to give a predicted PCR product of 388 nt. The sense primer was 5′-TCACCTCCCAATTCTGTGTAATCAAGA-3′, and the antisense primer was 5′-GATTATACCGCCTTCGTTCCTTAAACC-3′. PCRs were carried out with 100 nM primers for 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 54°C), and extension (2 min at 72°C).

General Methods. RNA was isolated by the RNAzol method (Biotecx Laboratories, Houston). DNA sequencing was done by the dideoxynucleotide technique (13) after subcloning of appropriate DNA fragments into M13.

RESULTS

Isolation of Clone HTm_4. By differential screening of the K562 hematopoietic library with cDNA probes from two hematopoietic libraries (enriched for hematopoietic cDNAs) and cDNA probes from the nonhematopoietic BS-1 library, clones that hybridized positively only to the hematopoietic probes were isolated. One of these, HTm_4, was used as a probe to screen a Northern blot panel consisting of total RNAs from various hematopoietic and nonhematopoietic cell lines.

Expression Pattern of HTm_4. A combination of Northern blot and RT–PCR analysis was used to determine the spectrum of tissue and lineage expression of the gene. The cDNA insert of clone HTm_4 hybridized to a transcript of about 1.7 kb in five hematopoietic lines which included myeloid and erythroid lineages and to normal human bone marrow cells (Fig. 1). The HTm_4 mRNA was not detectable in a T-cell lymphoma line (LY17) and in a lymphomyeloid leukemic line with T and granulocytic differentiation potential (DU528). In all seven nonhematopoietic cell lines—which included lung, cervical, brain, skeletal muscle, melanoma, hepatoma, and bone marrow stromal cells—no hybridizing mRNA could be detected. These nonhematopoietic lines included cells of ectodermal, endodermal, and mesodermal origin. The mRNA was also absent in the primary blast cells of a patient diagnosed to have M4 AML.

To facilitate screening of RNA samples, particularly those derived from cells in quantities too limited for Northern blot analysis, we examined expression by RT–PCR (Fig. 2). The quality of the first-strand cDNAs obtained after RT was satisfactory as evaluated by using primers for the housekeeping gene *HPRT* (data not shown). Based on nucleotide sequence of HTm_4 cDNA (see below), oligonucleotides were synthesized and used as specific primers for PCR amplification. The predicted PCR product, a 388-nt DNA, was obtained in normal bone marrow cells and the HL-60 cell line

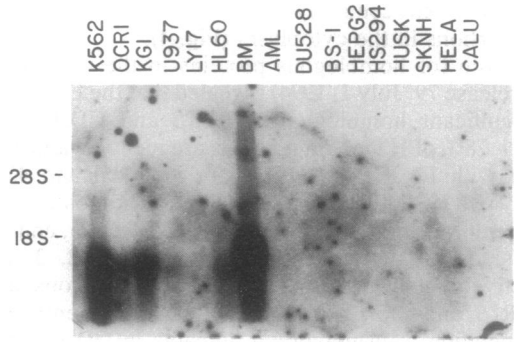

FIG. 1. Northern blot analysis of HTm_4 mRNA in human hematopoietic and nonhematopoietic cell lines and primary bone marrow (BM) and AML cells. Fifteen micrograms of total RNA was resolved in a denaturing agarose gel, transferred to a Hybond-N filter (Amersham), hybridized with ^{32}P-labeled HTm_4 cDNA probe, and washed with 0.2× standard saline citrate at 65°C before autoradiography for 24 hr at −80°C. Ethidium bromide staining of RNAs was used to monitor for equal loading of RNA (not shown).

but not in the HeLa and Ly17 cell line (Fig. 2), confirming the Northern blot analysis. RNAs from a human mast cell line (HMC-1) and a megakaryocytic line (MO7e) were also positive for HTm_4 mRNA. RNAs from normal eosinophils or neutrophils and eosinophils from a patient with HES also yielded the predicted PCR product. The low level of PCR product seen in the HES and neutrophilic sample was not used to evaluate the relative level of expression between cell types, since we did not perform quantitative PCR. Two leukemic T-cell lines (Ly13 and MOLT-4) and a myeloma cell line (OCI-My5) were also positive for HTm_4 (Fig. 2). All PCR-derived DNAs hybridized to radiolabeled HTm_4 in subsequent Southern analysis (data not shown).

Molecular Analysis of Human HTm_4 cDNA. The 1672-nt HTm_4 cDNA contains a long open reading frame, beginning at nt 97, encoding a 25-kDa protein of 214 aa (Fig. 3). Hydrophilicity analysis (15) revealed that HTm_4 contained four hydrophobic domains of 20–21 aa (Fig. 4). The N-terminal region before the beginning of the first hydrophobic domain contains four prolines. Each of the hydrophilic regions between the putative transmembrane segments contains a single proline. Several putative substrates for casein kinase 2 phosphorylation (16) of serine/threonine are found at residues 24 (TGPE), 155 (SSSE), 181 (TLLE), and 203 (SREE) and for protein kinase C phosphorylation at residue 149 (SLR). The sequence is consistent with a polypeptide chain that crosses the membrane four times, projecting two

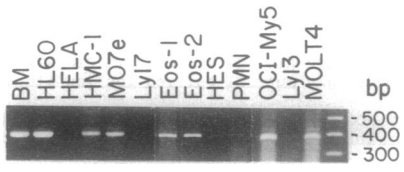

FIG. 2. RT–PCR analysis of HTm_4 mRNA in hematopoietic cell lines and primary hematopoietic cells. Thirty cycles of PCR amplification were performed. The specific PCR product for HTm_4 with the expected length of 388 bp is shown as an ethidium bromide-labeled band in a 1.3% agarose gel. The PCR product is not seen in the cervical cancer line (HeLa) and in a T-cell lymphoma line (Ly17), confirming Northern blot results in Fig. 1. PCR-amplified DNAs are seen in BM (bone marrow cells), HL-60 (promyelocytic cells), HMC-1 (mast cells), MO7e (megakaryocytes), Eos-1 and -2 (eosinophils), HES (eosinophils from a patient with HES), PMN (polymorphic mononuclear cells, neutrophils), OCI-My5 (multiple myeloma cells), Ly13 (T-cell lymphoma cells), and MOLT-4 (T-cell leukemia cells).

small loops extracellularly, and retaining the N- and C-terminal portions in the cytoplasm (Fig. 4).

A search for homologous proteins in the GenBank data base (release 79, July 1, 1994) revealed that the HTm_4 protein has significant homology to $Fc_\varepsilon RI\beta$ and CD20 (Fig. 5). Overall, 28% of HTm_4 amino acids are identical and 51% are similar with $Fc_\varepsilon RI\beta$, and 21% are identical and 45% are similar with CD20. The highest degree of homology is in the transmembrane domains, where 40%, 60%, 35%, and 33% of the residues in transmembrane domains 1, 2, 3, and 4, respectively, of HTm_4 are identical to the analogous domains in $Fc_\varepsilon RI\beta$, and 50%, 35%, 35%, and 24% are identical to the corresponding transmembrane domains in CD20. The four hydrophobic domains of HTm_4 are similar in size and placement to the four hydrophobic domains in $Fc_\varepsilon RI\beta$. In CD20 the first and second putative transmembrane domains form a continuous stretch of 55 hydrophobic amino acids without an obvious inter-transmembrane hydrophilic bridge. Similarities between the three proteins are also found in the N and C termini outside of the transmembrane region and in the inter-transmembrane domains, but at a lower level. For example, in the first 49 aa of HTm_4, 16% are identical to the N terminus of $Fc_\varepsilon RI\beta$ while 8% are identical to the corresponding region in CD20. Other interesting features derived from this comparison include the presence of several proline residues in the N termini of all three proteins, three of which may be aligned in identical position; two identical cysteine residues in the second extracellular domain between transmembrane domains 3 and 4; and a highly conserved serine/proline motif in the C end of all three proteins.

Expression of HTm_4 During Differentiation of Cell Line U937. To ascertain whether expression of the HTm_4 mRNA may vary with the stage of cellular differentiation, we examined the consequences of induced differentiation in the monoblastic cell line U937. Exposure of the cells to PMA rapidly induced differentiation to macrophages, as confirmed morphologically and molecularly by monitoring the marker for terminally differentiated macrophages, CD11b (17) (Fig. 6). Over a period of 48 hr, HTm_4 mRNA showed an initial increase but by day 3 was detected at only a very low level.

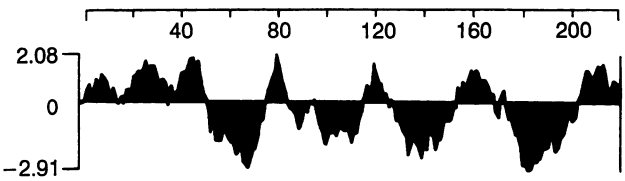

FIG. 4. Hydrophilicity plot of the predicted amino acid sequence for HTm_4 as analyzed with the Kyte–Doolittle algorithm (15). Amino acids are numbered above the plot. Positive values indicate hydrophilic regions, and negative values indicate hydrophobic regions.

The HTm_4 Gene Is Located on Chromosome 11q12–13. Forty-one chromosomes from 30 metaphases were scored for the positive chromosomal band. Band 11q12 was labeled on 18 chromosome 11 homologues, band 11q13.1 on 21 chromosome 11 homologues, and band 11q13.2 on 2 chromosome 11 homologues. No signal was detected on other chromosomes in these cells. Analysis of a metaphase is shown in Fig. 7. Similar results were obtained in an additional experiment using this probe. Thus, HTm_4 is localized to chromosome 11q12–q13.1.

DISCUSSION

HTm_4 appears to represent a hematopoietic cell-specific gene. The hematopoietic cell lines examined represent cells

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1    GTG ATC TTT TCT GAG TGT CTC CTA CTT GCG ACA AGG TGG ACT TGG GAG GAA AGC CGT CTG
61   CCA AAG CCT GAA GCC TCC AAG CCA TAA ACA ACC CCA ATG GCC TCC CAC GAA GTT GAT AAT
                                     met ala ser his glu val asp asn  8

121  GCA GAG CTG GGG TCA GCC TCT GCC CAT GGT ACC CCA GGC AGT GAG ACG GGA CCA GAA GAG
     ala glu leu gly ser ala ser ala his gly thr pro gly ser glu thr gly pro glu glu  28

181  CTG AAT ACT TCT GTC TAC CAC CCC ATA AAT GGA TCA CCA GAT TAT CAG AAA GCA AAA TTA
     leu asn thr ser val tyr his pro ile asn gly ser pro asp tyr gln lys ala lys leu  48

241  CAA GTT CTT GGG GCC ATC CAG ATC CTG AAT GCA GCA ATG ATT CTG GCT TTG GGT GTC TTT
     gln val leu gly ala ile gln ile leu asn ala ala met ile leu ala leu gly val phe  68

301  CTG GGT TCC TTG CAA TAC CCA TAC CAC TTC CAA AAG CAC TTC TTT TTC TTC ACC TTC TAC
     leu gly ser leu gln tyr pro tyr his phe gln lys his phe phe phe phe thr phe tyr  88

361  ACA GGC TAC CCG ATT TGG GGT GCT GTG TTT TTC TGT AGT TCA GGA ACC TTG TCT GTT GTA
     thr gly tyr pro ile trp gly ala val phe phe cys ser ser gly thr leu ser val val 108

421  GCA GGG ATA AAA CCC ACA AGA ACA TGG ATA CAG AAC AGT TTT GGA ATG AAC ATT GCC AGT
     ala gly ile lys pro thr arg thr trp ile gln asn ser phe gly met asn ile ala ser  128

481  GCT ACA ATT GCA CTA GTG GGG ACT GCT TTT CTC TCA CTA AAT ATA GCA GTT AAT ATC CAG
     ala thr ile ala leu val gly thr ala phe leu ser leu asn ile ala val asn ile gln  148

541  TCA TTA AGG AGT TGT CAC TCT TCA TCA GAG TCA CCG GAC CTA TGC AAT TAC ATG GGC TCC
     ser leu arg ser cys his ser ser ser glu ser pro asp leu cys asn tyr met gly ser  168

601  ATA TCA AAT GGC ATG GTG TCT CTA CTG CTG ATT CTC ACC TTG CTG GAA TTA TGC GTA ACT
     ile ser asn gly met val ser leu leu leu ile leu thr leu leu glu leu cys val thr  188

661  ATC TCT ACC ATA GCC ATG TGG TGC AAT GCA AAC TGC TGT AAT TCA AGA GAG GAA ATT TCC
     ile ser thr ile ala met trp cys asn ala asn cys cys asn ser arg glu glu ile ser  208

721  TCA CCT CCC AAT TCT GTG TAA TCA AGA ATA CCT CCT TAT GAA AAT AAT TCT GAG AGC ATG
     ser pro pro asn ser val END                                                    214

781  AAT ATT TGA CCT TAA ATC TCC AGT GAC TCA GAG CTT CAC CCA CAA ACT CAG GAG AAC ATA
841  AGC CTG CTC GTA AAG CTC AAT CCT TCT ATC ATG GCA CCA ATC ACA AGA ACC TTG GAC GTT
901  TGA CTG ACT CTA TCC TTT CTC TCC TAA CTA TAA ATC CTA TTT GTG TGT CGT GGG TAT GGA
961  AGG ACA GAT ATA TTT CTT TAG GCA TTC TTG GAT ATC TGT AAC TTC TAT GAT CAT TAC TCC
1021 AAA GTT GTT TCC AGA AAT TGG TTC TAT TTC TTC TTA TCC ACC TAC TCC ATT GCT TTA TGA
1081 GGT TTA AGG AAG GAA GGC GGT ATA ATC CCT ATT CAA TAT ATT TTT TCT AAA ATC CAA CTT
1141 CTG ACC GCC CAG TAG GAA GAA AAA TGA GAC ATT TTT TCC ATT ACA GAG AAA TGC TTC TTG
1201 ACT TTA ACA TCA GCA TTA TAA AAA GTG TCA AAT AAA AAA TTA CCA TCA TTA TCA TTA AAA
1261 TAA ATT TTC ACT GTA TTT GAG ATG GGA GGG TTA AGG CTC AGG GAT TTT ATT TCA GTG AAC
1321 TGC TGG AAC TCA CAC ATG CCC TGA TAT GTA AAT GAT GAT TTA TGT TGG CGA GTC TGA GAG
1381 CAA GCC CAA ATG TGT TCT TCA AAG GAC AAT GGG AAA CTG TAA AGT AGA GAA CTA AAG AAT
1441 AAG GCC TIT AGA ATC TGA CAC ATC TGG GTT CAA ATT CTG AAA CTG TCA CTT ATT ACC TGT
1501 ATG AAC ATG GGC AAA TTA TCT AAT CTC TCT GAT CTA TTT TTC CTC ATC TGT AAA ATA GGT
1561 GTA ATA ATA ACA ACT ACT TTG TCG GTT GCT CTG AGG GTT AAA TGA AAA TAAAAA GAA AAT
1621 GTG AAA CAG CAC CAC AGG TAC TTG AAA AAA AAA AAA AAA AA
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FIG. 3. Nucleotide and deduced amino acid sequences of clone HTm_4. Nucleotide sequence is numbered at left. The amino acid sequence of the longest open reading frame is numbered at right beginning with the first (presumed initiating) methionine. An upstream in-frame stop codon, TAA, is indicated in bold letters at position 85. A TAA stop codon (END) is followed by a 3′ untranslated region containing an AATAAA polyadenylylation signal. The four putative transmembrane domains are underlined. The two putative phosphorylation sites are underlined with dotted lines.

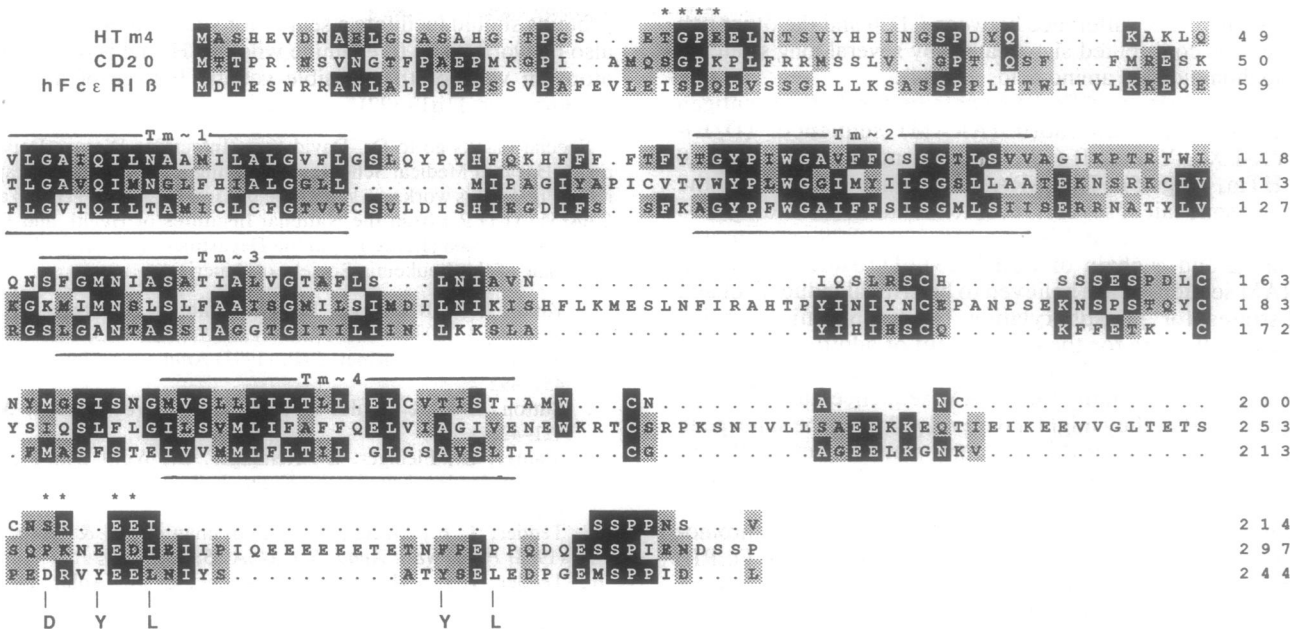

FIG. 5. Amino acid sequence of HTm_4 protein is compared with that of human CD20 and human $Fc_\varepsilon RI\beta$ proteins. Sequences for CD20 and human $Fc_\varepsilon RI\beta$ were obtained from the Swiss-Prot data base. Gaps between residues are marked by dots. Residues identical or conserved in two or more proteins are shaded. White amino acid residues on a black background correspond to identical amino acids; black amino acid residues on a gray background represent groups of conservative amino acid replacements as follows: S,T,G,A,P; L,M,I,V; E,D,Q,N; R,K,H; F,Y,W; C. The four putative transmembrane domains for HTm_4 and $Fc_\varepsilon RI\beta$ are marked (Tm) and indicated by lines above and below the respective residues. Tm-1 and Tm-2 for CD20 stretch as a continuous hydrophobic domain, residues 51–103. The antigen receptor activation motif (ARAM) DYLYL in $Fc_\varepsilon RI\beta$ is indicated. The two putative phosphorylation sites for HTm_4 are marked by stars.

at various immature stages of differentiation. An induction experiment with U937 promonocytic cells showed that in some lineages, HTm_4 may be downregulated upon terminal differentiation.

The predicted structure of HTm_4 demonstrates the relation of this protein to $Fc_\varepsilon RI\beta$ and the CD20 antigen (Fig. 5) and provides evidence for a family of four-transmembrane proteins. The conservation of amino acids in all three proteins is highest in the four transmembrane domains. While much greater divergence exists in the hydrophilic N and C termini, several amino acids within these regions are conserved, such as four or five prolines in the N terminus of all three proteins. Two conserved cysteine residues in the second extracellular domain between transmembrane regions 3 and 4 suggest that intra- or intermolecular disulfide bonds in this domain are essential to all three proteins. HTm_4 also contains two putative phosphorylation sites (Thr^{24} and Ser^{203}) in the cytoplasmic region of the protein. Finally, there is a well-conserved SP(P) motif near the C end of all three proteins whose significance

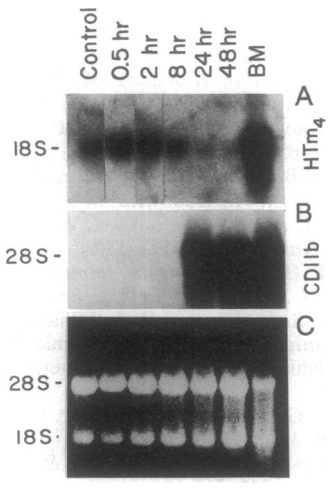

FIG. 6. Northern blot analysis of HTm_4 mRNA from U937 cells before and after induction with PMA to undergo differentiation into macrophages. (*A*) Time course of HTm_4 after induction of U937 with PMA. The left lane (Control) contained RNA from U937 cells collected just prior to PMA treatment. BM, RNA from bone marrow cells, is a positive control. The positions of the 18S and 28S rRNAs are indicated at left. (*B*) The same blot as in *A* was probed for CD11b mRNA as a marker for proper induction of differentiation. (*C*) Gel corresponding to the blot in *A* and *B*, after ethidium bromide treatment. The amount of RNA in each lane was constant, except in lane BM, where 10 μg instead of 20 μg of total RNA was loaded.

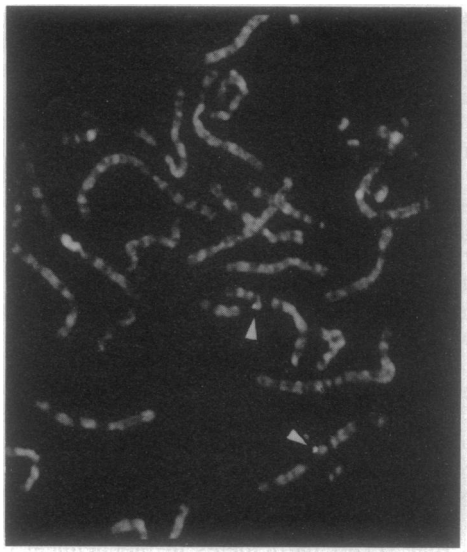

FIG. 7. Localization of HTm_4 to chromosome 11q12–13.1 by FISH. The chromosome 11 homologues are identified in the partial metaphase with white arrowheads; specific labeling is seen at 11q12–13.1. Separate images of the 4′,6-diamidino-2-phenylindole-stained chromosomes and the hybridization signal were acquired and merged by using the NIH IMAGE 1.52 software.

is unknown. The difference between CD20 and the other two proteins is contributed significantly by several long stretches of nonhomologous amino acids (Fig. 4).

The C terminus of $Fc_\varepsilon RI\beta$ contains the Reth or antigen receptor activation motif (ARAM) sequence, (D/E)-$X_2YX_2LX_{6-8}YX_2$(L/I) (18), which is not present in CD20 or HTm_4 (Fig. 5). The ARAM sequence is found in the cytoplasmic tail of several receptor subunits, including CD3 γ, δ, ε, and ζ, Igα, and Igβ, in MB-1 and B29 antigen, and in the β and γ chain of $Fc_\varepsilon RI$ (19). Tyrosine residues in ARAM sequences are believed to be critical inducers of and substrates for phosphorylation by cytoplasmic tyrosine kinases, allowing for the recruitment of additional effector molecules (19–21). The common exon/intron organization of the genes containing the ARAM sequence has led to the suggestion that they might have evolved from the same gene family (19). However the structural similarity of $Fc_\varepsilon RI\beta$ to CD20 and HTm_4 suggests that the ARAM sequence was acquired by the $Fc_\varepsilon RI\beta$ gene during evolution.

Our mapping by FISH localizes the HTm_4 gene to chromosome 11q12–13.1, the same location as the CD20 and $Fc_\varepsilon RI\beta$ genes (22). Both the murine $Fc_\varepsilon RI\beta$ gene and the murine equivalent for CD20, Ly-44, are located in the same position on mouse chromosome 19 (6, 8). Therefore it appears that the three genes originated and evolved from the same locus, further supporting our proposition that they are members of the same family of related proteins. They also form a family of proteins that is quite distinct from another large family of four-transmembrane proteins related to TAPA-1 (23, 24) which includes CD9, CD37, CD53, CD63, and R2.

The identification of a gene product such as HTm_4 related to $Fc_\varepsilon RI\beta$ is significant for two reasons. (*i*) The exact importance of the β subunit in $Fc_\varepsilon RI$-expressing cells is not clear. While simultaneous cotransfection of the α, β, and γ genes is necessary to induce surface expression of murine $Fc_\varepsilon RI$, cotransfection of the human α and γ genes without the β gene is sufficient to induce expression of high-affinity Fc receptors (25). Furthermore, functional high-affinity IgE Fc receptors may be found on monocytes in the absence of the β chain (4). A reasonable question is whether other subunits are involved. (*ii*) An emerging theme is the diverse association of subunits in Fc receptors of different hematopoietic cells. For example, $Fc_\varepsilon RI\beta$ was found to be associated with the low-affinity Fc receptor for IgG, $Fc_\gamma RIII$ (CD16), in mast cells (26). $Fc_\varepsilon RI\gamma$ has also been found as a homodimer in association with $Fc_\gamma RIII$ in macrophages (27) or as a heterodimer with ζ and η chains in the T-cell receptor complex (28). In natural killer cells, $Fc_\varepsilon RI\gamma$ may be found as homodimers and as a heterodimer with the ζ chain of the T-cell receptor (29). The γ chain can also form an association with the high-affinity receptor for IgG, $Fc_\gamma RI$ (CD64), in monocytic cell lines and neutrophils (30, 31). These findings suggest that a variety of signal transduction complexes composed of different subunits might mediate similar effector functions but with different functional consequences. Association of these subunits with alternative ligand-recognition subunits in a multimeric receptor complex would allow coupling of distinct ligands to common signaling pathways.

Thus, it would be important to determine whether HTm_4 may function, like $Fc_\varepsilon RI\beta$, as a subunit in some currently known receptor complexes or in other, as yet unidentified receptors. The expression of HTm_4 in all hematopoietic lineages and not in any of the nonhematopoietic cells tested indicates that HTm_4 participates in biochemical pathways unique to hematopoietic lineages.

It is possible that other related members of this family of transmembrane proteins exist. Our identification of the regions of highly conserved amino acids among all three proteins should facilitate a search for such proteins. It should also be worthwhile to examine whether HTm4 is involved in atopy in view of the possible genetic linkage of atopy to chromosome 11q13 (22).

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