A Novel Alternative Spliced Chondrolectin Isoform Lacking the Transmembrane Domain Is Expressed during T Cell Maturation*

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Chondrolectin (CHODL) is a novel type I transmembrane protein containing one carbohydrate recognition domain (CRD) of C-type lectins. Recently, database searching revealed a variant of CHODL (AK022689) with a different 5′ leader sequence derived from a new putative upstream alternative promoter (P2). The P2 promoter gives rise to at least three alternatively spliced isoforms, designated as CHODLp, CHODLtf, and CHODLsf. Of all variants, the alternative exon E-splicing isomers (CHODLtE/CHODLsE) are expressed exclusively in the T lymphocyte lineage and are regulated during T lymphopoiesis. Peripheral T lymphocytes demonstrated a unique exon E-splicing pattern in comparison with end maturation stage thymocytes, suggesting its association with the post-thymic maturation of T cells. Since exon E encodes the transmembrane domain of CHODL, the exon E-skipping variant results in a non-transmembrane domain-containing isoform (CHODLtE/CHODLtE) terminating in the QDEL sequence, thus suggesting different functional attributes of CHODL isoforms during the development of T cells. Double label immunofluorescence experiments demonstrated that the transmembrane-containing isoform (CHODLp) colocalizes with rBet1 to the endoplasmic reticulum-Golgi apparatus. In summary, this study describes the molecular characterization of novel members of the chondrolectin family associated with T cell maturation and a subcellular localization of CHODLp in the endoplasmic reticulum-Golgi apparatus.

C-type lectins of animals are the most diverse family of animal lectins (1). They are generally multidomain proteins in which carbohydrate recognition domains (CRDs) provide calcium-dependent sugar-binding activity. The C-type CRD has a common sequence module of 115–130 amino acid residues containing four cysteines that are perfectly conserved and involved in two disulfide bonds. Those C-type CRD domains have been found in various kinds of proteins such as hepatic asialoglycoprotein receptor (2), lymphocyte Ig E receptor (3), mannose-binding protein (4), selectin (5), and proteoglycan core protein (6).

In general, animal lectins have a wide variety of functions. The principal function is to act as recognition molecules within the immune system and includes complement activation, recognition, and trafficking within the immune system, immune regulation (suppression or enhancement), and prevention of autoimmunity (1). Perhaps the best established functions outside the immune system are lysosomal enzyme transport by the phosphomannosyl receptors and the molecular chaperone role of calreticulin in the endoplasmic reticulum (7, 8).

Recently, we have described the isolation and characterization of a novel C-type lectin, designated as chondrolectin (9). The mouse chondrolectin gene (chold) was first isolated from a subtracted library derived from tails of 4-day-old mice and therefore referred to as chondrolectin. The mouse and human gene structures are very similar, consisting of six exons and five introns (9, 10). In both species, the open reading frame for chondrolectin encodes a type I membrane-associated polypeptide of 273 amino acids, containing an N-terminal signal sequence, a single CRD, a transmembrane region (TM), and an intracellular C terminus. Chondrolectin is structurally related to hamster layilin, a hyaluronan receptor (11, 12). However, chondrolectin does not contain the functional talin binding site located in layilin, a hyaluronan receptor (11, 12). Therefore, chondrolectin is not likely to have the function of a hyaluronan receptor. The distribution of chondrolectin gene expression in normal human tissues is in general very low. RT-PCR analysis revealed preferential expression of chondrolectin in testis, prostate, and spleen, whereas immunohistochemical analysis demonstrated that the expression is mainly limited to vascular muscle of testis, smooth muscle of prostate stroma, heart muscle, skeletal muscle, crypts of small intestine, and red pulp of spleen (9).

Although the physiological function of chondrolectin remains unknown at present, the identification of novel T cell-associated lectins such as layilin and chondrolectin provides new avenues for the study of the molecular mechanisms involved in the regulation of vascular muscle function and its response to immune system stimulation.
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ated isoforms of chondrolectin is of particular interest, because many proteins that we regard as recognition or adhesion molecules within the immune system are now known to be lectins. For example, CD44 is a hyaluronate-related C-type lectin and plays a role in lymphocyte recirculation; impaired homing to lymph nodes and thymus has been found in gene-targeted CD44-deficient mice (13).

In this study, we have identified three new members of the chondrolectin family that result from alternative promoter usage. All splicing variants described in the present study are derived from the distal alternative promoter and are differentially expressed in the T lymphocyte lineage. Two variants are devoid of the transmembrane domain (exon E of the human gene) and terminate in QDEL, a short sequence that is consistent with the motif for the endoplasmic reticulum retention signal. The transmembrane-containing isoform is localized in the ER/Golgi apparatus and is associated with T lymphocyte immaturity. Our results suggest that the alternative promoter usage could be a key mechanism controlling the expression of different chondrolectin isoforms with different biological function in T cell development.

EXPERIMENTAL PROCEDURES

Cell Lines and Antibodies—U937, a human histiocytic lymphoma cell line, and K562, a human erythroid-myeloid leukemia cell line, were used as nonlymphoid cell sources. RAJI, a human Burkitt's lymphoma cell line, was used as a source of B lymphocytes. JURKAT, MOLT-3, and HSB-2, human T cell lines derived from severe lymphoblastic leukemia, and SUP-T1, a human T cell line derived from a T cell lymphoblastic lymphoma, were used as a source of human T cells. Cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and cultured following the guidelines of the ATCC.

Fluorescin isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulins (Fab')2, phycoerythrin (PE), and allophycocyanin (APC) were as well as isootype-matched control antibodies were purchased from Dako (Glostrup, Denmark). Supernatant of the 43A1 hybridoma (IgG3; kindly donated by Dr. H. J. Bühring (University of Tubingen, Germany)) was used as a source of anti-CD34 antibody (14). All other monoclonal antibodies and conjugates (FITC, phycoerythrin (PE), and allophycocyanin (APC)) as well as isotype-matched control antibodies were purchased from Becton Dickinson (Erembodegem, Belgium). Mouse γ-globulins were obtained from Jackson Immunoresearch Laboratories (West Grove, PA). Antibodies (anti-β2-m, anti-LAMP1, and anti-KDEL) used for subcellular localization were purchased from Stressgen (Victoria, Canada). The anti-GFP monoclonal antibody (Sigma) and the anti-dsRED monoclonal antibody (Clontech) were used for Western blot analysis.

Isolation and Preparation of Hematopoietic Cells—Bone marrow samples were aspirated by sternal puncture from hematologically normal patients undergoing cardiac surgery. Cells were collected into sterile thin-walled tubes containing Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (FCS) and heparin (100 units/ml; Novo Nordisk, Bagsvaerd, Denmark), and the mononuclear cells were isolated by density gradient centrifugation over lymphocyte separation medium (ICN Biomedicals, Costa Mesa, CA). Samples were obtained after informed consent according to the guidelines of the Medical Ethics Committee of the Antwerp University Hospital. Postnatal thymus was obtained from children 0–12 years of age undergoing corrective cardiac surgery. Those samples were obtained and used following the guidelines of the Medical Ethical Commission of the University Hospital of Gent. Single cell suspensions were obtained by gentle disruption of the thymus in IMDM plus 10% FCS. Adult peripheral blood leukocytes were obtained after removing the red blood cells from a blood sample of a hematopoietic progenitor by the Lymphocette isolation procedure.

Flow Cytometric Cell Sorting—Freshly prepared adult bone marrow mononuclear cells were resuspended in IMDM at 105 cells/ml and incubated with 43A1 supernatant in a 1:10 dilution for 20 min at 4 °C, washed twice in IMDM supplemented with 10% FCS, and incubated with rabbit anti-mouse FITC (1:50 dilution) for 20 min at 4 °C. After washing twice in IMDM plus 10% FCS, the cells were incubated with mouse anti-CD34 PE (Becton Dickinson) and with a water-cooled argon ion laser (Coherent Innova Enterprise Laser), tuned to 488 nm at 170-milliwatt power. Cells with a low to medium forward scatter and a low side scatter, highly positive green (CD34) fluorescence, and an orange (CD38) fluorescence signal lower than the mean fluorescence plus 2 S.D. values of cells labeled with an irrelevant isotype-matched control antibody were retained as CD34+ CD38+ cells; cells with an orange fluorescence above this threshold were retained as CD34+ CD38− cells.

CD34+ thymocytes were purified by positive selection with MACS beads (Miltenyi Biotec, Auburn, CA), stained with CD1-FITC and CD34-APC, and sorted for CD34+ CD1− or CD34+ CD1+ progenitor cells by flow cytometry. To obtain immature single positive CD4 thymocytes, cells were stained with CD3-FITC and CD8-FTX and depleted with sheep anti-mouse IgG-coated beads (Dynabeads; Dynal AS, Oslo, Norway). The depleted cells were labeled with CD4+PE and sorted for CD4+ CD8− thymocytes. To obtain CD8+ thymocytes, CD4−CD8− thymocytes, and CD4+CD8+ thymocytes, cells were stained with CD8-FTX, CD4-FITC, and CD34-APC and subsequently sorted for the different populations.

Lymphocytes, monocytes, and granulocytes were sorted from peripheral blood based on the typical light scattering properties of the respective populations (i.e. low forward and orthogonal, intermediate forward and orthogonal, and intermediate forward and high orthogonal light scattering, respectively). T and B lymphocytes were further separated by fluorescence activated cell sorting after staining for CD3 with anti-CD3-FITC or CD19 (with anti-CD19-PE).

The purity of the sorted cell populations was always checked and was at least 98%.

PCR, Southern Blot, and RT-PCR—The expression from the distal and proximal promoters was analyzed using PCR and subsequent Southern blot analysis. Primer sets for transcripts of the distal and proximal promoters were designed. The S1 primer set, derived from the human CHODL cDNA (AF257472), was used for analyzing the expression of the proximal promoter: forward, 5′-ctagcactgtcctggttgactgag; reverse, 5′-tagcactttcagagactgag. The S2 primer set, derived from the human CHODL homologue (AK022689), was used for analyzing the distal promoter: forward, 5′-tagcactgtcctggttgactgag; reverse, 5′-tagcactttcagagactgag. First strand cDNAs of multiple human tissues (Clontech) were used as templates. The temperature profile for both PCRs was as follows: 95°C for 1 min and 36 cycles of 94°C for 10 s, 60°C for 10 s, and 72°C for 1.5 min. The final cycle included an extension at 72°C for 10 min. Resulting PCR products were fractionated on 1% agarose gel and transferred to a Hybond-N membrane, and subsequent Southern blot hybridization was performed according to standard procedure (15). Probes for hybridization were PCR amplitons derived from primer sets S1primer sets, respectively. S1primers were as follows: forward, 5′-gtcctgtgaggtgagatc; reverse, 5′-gtcctgtgaggtgagatc. The S2 primer set, derived from the human CHODL human homologue (AK022689), was used for analyzing the distal promoter: forward, 5′-tagcactgtcctggttgactgag; reverse, 5′-tagcactttcagagactgag. First strand cDNAs of multiple human tissues (Clontech) were used as templates. The temperature profile of the PCR reaction was as follows: 95°C for 3 min and 36 cycles of 94°C for 10 s, 50°C for 30 s, and 72°C for 1.5 min. The final cycle included an extension at 72°C for 10 min.

The exon E-splicing pattern in multiple human tissues was analyzed using a primary PCR and nested PCR. The primers used were derived from the exon D and exon E of human CHODL separately: forward, 5′-tagcactgtcctggttgactgag; reverse, 5′-acagctactacctgaa. First strand cDNAs of multiple human tissues (Clontech) were used as templates. The temperature profile of the PCR reaction was as follows: 95°C for 3 min and 36 cycles of 94°C for 10 s, 50°C for 30 s, and 72°C for 1.5 min. The final cycle included an extension at 72°C for 10 min.

The exon E-splicing pattern in human hematopoietic cell lines (HSB-2, SUP-T1, JURKAT, MOLT3, K562, RAJI, and U937) was analyzed using RT-PCR and subsequent nested PCR. First strand cDNA synthesis was primed by oligo(dT)12–18, and catalyzed by the RNase H derivative of Moloney murine leukemia virus reverse transcriptase (Invitrogen) after poly(A) RNA was extracted and selected using the Quickprep™ micro-mRNA purification kit (Ambion, Biosciences). The PCR of subsequent nested PCR were carried out as described above.

To more precisely define the expression of exon E-splicing variants in the K562, RAJI, and U937 cell lines, an RT-PCR analysis of the human β-actin mRNA (as the internal reference template) was performed. The primers used were as follows: forward, 5′-ctagcactgtcctggttgactgag; reverse, 5′-acagctactacctgaa. The temperature profile of the PCR reaction was as follows: 95°C for 3 min and 36 cycles of 94°C for 10 s, 60°C for 30 s, and 72°C for 1 min. The final cycle included an extension at 72°C for 10 min.

All PCRs were carried out in 200-μl thin-walled tubes using a
Cloning and Sequencing of CHODL Variants from Leukocytes—CHODL variants of leukocytes were amplified using PCR and nested PCR. Primer sets (S1 and S2) and PCR conditions were described above. Nested PCR products were fractionated on a 1% agarose gel and stained with ethidium bromide. Amplicons were visualized under UV light. Distinct bands were excised and subsequently cloned into pCR-script plasmid according to the manufacturer’s instructions (Stratagene, La Jolla, CA). Clones designated as pUIA847, pUIA848, and pUIA849 were sequenced using the dye terminator method (Amersham Biosciences) by an automated ABI type 373 DNA sequencer (Applied Biosystems).

Construction of Fluorescent Protein-tagged CHODL Isoforms—Isoforms of CHODL were separately tagged with in-frame sequences of red fluorescent protein (RFP) and GFP. The coding sequences of variants were amplified from cloned splice variants. The primers used were as follows: forward, 5'-gtcggagcatctctggtatgctctcttttgga or 5'-tttgaagatggtgcttgagtgcttttggga; reverse, 5'-agttcagctataaaactctcttctctctctctcttctctctctctctc. The reverse primer contains 27 in-frame nucleotides encoding a hemagglutinin epitope atcaggaacatcataaggatacagttcatcttgcagatcctttgtg. The reverse primer was excised with plasmid (Stratagene, La Jolla, CA). After confirmation of sequences, the amplicons were subcloned into the pCR-Script sRed1-N1/pEGFP-N1 vectors (Clontech). The resulting constructs were subsequently ligated into the same restriction enzyme-digested pD-CHODL

Results
Evidence for Transcription Initiation Upstream of the CHODL P1 Promoter—Data base (GenBankTM/EMBL) searching with the CHODL sequence revealed several homologues (Nivelles, Belgium).

Fig. 1. Organization of the human CHODL genome and mechanisms for the generation of isoforms. The genomic structure of the human CHODL gene is shown; the characterized exons are indicated with boxes. The arrow with open head indicates the proximal promoter (P1), whereas the arrow with open head represents the distal promoter (P2). The single arrowheads indicate the site of primer set (S1) for the expression of the proximal promoter, whereas the double arrowheads represent the site of primer set (S2) used to study the expression of the distal promoter. Three-head arrows indicate the site of primer set used for the analysis of exon E splicing pattern. Boxes with capital letters denote exons of CHODL, and boxes with numbers denote exons upstream of the proximal promoter. The diagrams above and below the genomic structure illustrate the contributions of various exons to the indicated CHODL isoforms.

tein of 232 amino acids (aa), since its start codon corresponds to the second ATG of CHODL, resulting in an isoform lacking the 41 first amino-terminal aa of CHODL. Analysis of its nucleotide sequence and comparison with the human genomic DNA sequences (16) of chromosome 21 revealed that AK022689 is derived from a promoter region 330 kb upstream of the CHODL P1 promoter. The existence of this transcript provides evidence for the usage of an alternative promoter for the CHODL gene (Fig. 1). Due to the low expression of CHODL, the expression from the proximal (P1) and distal (P2) promoters was analyzed using PCR and subsequent Southern blotting.

Specific expression from the proximal (P1) promoter was detected in human adult colon, small intestine, thymus, and pancreas (Fig. 2A); specific expression from the distal (P2) promoter was detected in human adult leukocytes and placenta (Fig. 2B). Expression from both proximal (P1) and distal (P2) promoters was found in human adult prostate, spleen, and testis (Fig. 2, A and B). Neither expression from the distal promoter nor expression from the proximal promoter was detected in human adult ovary, brain, heart, liver, lung, and muscle. We detected significant molecular heterogeneity of the PCR products derived from the distal promoter (Fig. 2B). Subsequent cloning and sequencing of these PCR products revealed that this heterogeneity is the result of alternative splicing, suggesting the presence of variable CHODL isoforms.

Characterization of CHODL Variants Expressed in Leukocytes—As noted above, we detected molecular heterogeneity in RT-PCR amplicons, suggesting the presence of different CHODL isoforms. This hypothesis was further tested by PCR analysis using primers flanking exon E, a putative transmembrane domain of CHODL. As shown in Fig. 3, this primer set amplified a PCR product (smaller than the expected one) unique for leukocytes, suggesting the existence of an alternative exon E-splicing variant. PCR and subsequent nested-PCR using primer sets (S2 and S3) amplified three distinct ampli cans from cDNA of leukocytes. Subsequent cloning and sequencing analysis revealed that they represent three novel variants of CHODL. The nucleotide sequence of these cDNAs has been submitted to GenBankTM/EMBL data base with ac-
Exon E is located within the coding region of CHODL specific expression from the proximal promoter (Fig. 2A) and from the distal promoter (Fig. 2B) are shown. A and B). The third novel variant, CHODL, does not contain exon 3 and skips exon E (Fig. 1). We failed to isolate 5'-rapid amplification of cDNA end clones of these cDNAs and are not in a position to define the precise start of transcription and to determine whether or not they contain exon 1 of AK022689. Since the 5' leader sequences of these novel variants contain an in-frame stop codon without additional in-frame ATG, variants with the insertion of exon 3 still share the same translation initiation site in exon B as variant AK022689 (i.e. the second ATG of the CHODL gene). Exon E is located within the coding region of CHODL and encodes the putative transmembrane region (9). Therefore, alternative splicing of this exon results in a non-transmembrane-containing isoform of CHODL with an altered carboxy-terminal sequence due to a frame shift (Fig. 4, A and B). The non-transmembrane-containing isoforms or soluble isoforms (designated as CHODLs/CHODLs) consist of 236 aa with an approximate molecular mass of 27.38 kDa and a pI of 4.8 (see, on the World Wide Web, us.expasy.org/cgi-bin/protparam) (Fig. 4B). A profile searching on ScanProsite (available on the World Wide Web at us.expasy.org/cgi-bin/scanprosite) revealed that CHODLs/CHODLs contains a CRD motif (aa 1–138), three N-glycosylation sites (aa 45–48, 199–202, and 220–223), two protein kinase C phosphorylation sites (aa 8–10 and 100–102), two casein kinase II phosphorylation sites, and one endoplasmic reticulum retention sequence (QDEL) at the very C terminus end (aa 233–236).

Alternative Exon E Splicing in Hematopoietic Cells—Leukocytes are a heterogeneous population of hematopoietic cells. In order to define more precisely the cellular site of alternative exon E splicing, we performed a more detailed RT-PCR analysis on subsets of peripheral blood leukocytes. Four subsets of peripheral blood leukocytes (peripheral T lymphocytes, B lymphocytes, monocytes, and granulocytes) were sorted by flow cytometry. Their exon E-splicing pattern was analyzed using RT-PCR. The exon E-containing variant and exon E-skipping variants were discriminated based on the size of their amplons. As shown in Fig. 5A, B lymphocytes express the exon E-containing variants only; peripheral blood T lymphocytes (CD3+CD4+CD8- and CD3+CD8+CD4+ cells) express exon E-skipping variants only; granulocytes and monocytes express neither exon E-containing nor exon E-skipping variants. Since the expression of the exon E-skipping variants is restricted to T lymphocytes, we investigated the expression of the exon E-splicing variants during the development of T lymphocytes. Bone marrow progenitor cells (immature CD34+CD38- cells and more mature CD34+CD38- cells) express the exon E-containing variant only. Thymocytes at different stages of maturation (CD34+CD11a- cells, CD34+CD11a+ cells, immature single CD4+ cells, double positive CD4+CD8+ cells, and single CD8+ cells) express the exon E-containing variant predominantly and the exon E-skipping variants slightly. Single mature CD4+ and more mature thymocytes express only the exon E-containing variant. Peripheral blood T cells (CD3+CD4+CD8- cells or CD3+CD8-CD4- cells) express only the exon E-skipping variants (Fig. 5B). The differential utilization of exon E-splicing variants intra- and extrathymus suggests their association with the differentiation of T lymphocytes. In accordance with its T cell origin, HSB-2, SUP-T1, JURKAT, and MOLT-3 cells express the exon E-skipping variants. The presence of the exon E-containing variant is compatible with the immature stage of those leukemic cells. The exon E-containing variant is present...
in RAJI cells, a B-cell line, and in K562, a erythromyeloid cancer cell line. The exon E-containing variant is absent in the monocyte-derived cell line U937 (Fig. 5).

Subcellular Localization of CHODLf/CHODLfE Isoforms—To probe the biological function of CHODL variants, we analyzed the expression and subcellular localization in transfected COS1 cells. The RFP-tagged exon E-containing isoform (RFP-CHODLf) and GFP-tagged exon E-skipping isoform (GFP-CHODLfE) were constructed separately and subsequently used for transfection of COS1 cells. Western blot analysis revealed both fusion proteins as a single band of similar molecular mass (~85 kDa) (Fig. 6). To determine the subcellular localization of both isoforms, we performed immunofluorescence microscopy. In all cells in which fusion protein was detected, RFP-CHODLf (Fig. 7A) appeared as distinct ringlike structures, localized at the perinuclear region and/or along the extension of cells, whereas the GFP-CHODLfE isoform revealed a diffuse distribution in the cytoplasm (Fig. 7B). In order to evaluate whether a fluorescent protein tag can interfere with the localization of proteins to which it is fused, we subsequently used a small FLAG epitope-tagged CHODLf construct in a double label immunofluorescence experiment. Both constructs, FLAG-tagged CHODLf and RFP-CHODLf, were overexpressed in COS1 cells (Fig. 7C). The coincidence of both proteins in the same cells is clearly visible and indicated by yellow staining. This demonstrates that the localization of RFP-CHODLf is not affected by RFP. To more precisely map the subcellular localization of the CHODLf isoform, co-immunostaining experiments were performed with ER, Golgi, mitochondrion, and lysosome markers, respec-

Fig. 4. Deduced amino acid sequences of novel CHODL isoforms. A, CHODL, CHODLf, and CHODLfE/CHODLfE are shown schematically. SS, in-frame shift sequence. SP, signal peptide. B, the deduced amino acid sequences of CHODLfE/CHODLfE. Letters in italics represent the amino acid sequence (aa 1–138) that is part of the CRD of CHODLfE/CHODLfE. The arrowhead indicates the site of exon E skipping. Gray shading, frame-shifted sequence due to exon E skipping. Potential N-glycosylation sites in the frame-shifted sequence are underlined. The asterisks indicate the putative retention signal for the endoplasmic reticulum.

Fig. 5. Alternative exon E splicing in hematopoietic cells. Poly(A)⁺ RNA was extracted from FITC-sorted hematopoietic cells and cell lines. First strand cDNA synthesis was primed with oligo(dT)₁₂₋₁₅ and catalyzed by the RNase H⁻ derivative of Moloney murine leukemia virus reverse transcriptase. The subsequent PCR and nested PCR were performed using primers flanking exon E as described under “PCR, Southern Blot, and RT-PCR.” PCR products were fractionated on a 1% agarose gel and visualized by ethidium bromide staining. The 866-bp amplicon is derived from exon E-containing variants, whereas the 764-bp amplicon is derived from exon E-skipping variants. Alternative exon E splicing patterns in subsets of leukocytes (A), during T lymphopoiesis (B), and hematopoietic cell lines (C) are shown. Images are representative for the experiment performed in duplicates.
In-frame ATG. The skipping of exon E results in a non-
sequence, because in-frame sequences of exon 3 do not contain
taining and exon E-skipping variants. Hematopoietic progenitor
ping cells, and intermediate cells (expressing both exon E-con-
dominate exon E-containing cells, predominant exon E-skip-
ating exon E splicing pattern in
brane isoforms in T lymphocytic cells. The expression of both
expression pattern of these variants, particularly to the expres-
specificity of the CRD.
contains the whole carbohydrate recognition domain. This indi-
forms that we identified in T cells lack the signal peptide but
frame-shifted tail ending with the sequence QDEL. All iso-
and thus support the hypothesis mentioned above. A more
transcripts renders variants that differ either in the 5'-un-
translated region or coding region, thus resulting in a different
protein translation efficiency or different proteins. In the pres-
complex splicing patterns
generated during the transcription of the chondrolectin gene
ent primer sets probably reflects complex splicing patterns
during the transcription of the chondrolectin gene
and thus support the hypothesis mentioned above. A more
extent characterization of P2-derived transcripts will be
ecessary in order to understand the modulation of transcription
of the chondrolectin gene.
In the present study, we have identified three novel CHODL
splice variants (CHODLp, CHODLAE, and CHODLSEG) derived
from the P2 promoter and expressed in T cells. These variants
are attributed to alternative splicing of exon 3 and exon E,
either by insertion of an additional exon (exon 3 for CHODLp) or
by skipping of exon E (CHODLAE) or by a combination of both
(CHODLSEG). Insertion of exon 3 does not change the protein
sequence, because in-frame sequences of exon 3 do not contain
an in-frame ATG. The skipping of exon E results in a non-
transmembrane-containing or soluble isoform of CHODL with
a frame-shifted tail ending with the sequence QDEL. All iso-
forms that we identified in T cells lack the signal peptide but
contain the whole carbohydrate recognition domain. This indi-
cates that these isoforms share the same ligand binding spec-
ificity of the CRD.

One of the striking findings in this study relates to the
expression pattern of these variants, particularly to the expres-
profile of the transmembrane-containing/nontransmem-
brane isoforms in T lymphocytic cells. The expression of both
isoforms was analyzed by RT-PCR using a common primer set
flanking both sides of exon E. The exon E splicing pattern in
hematopoietic cells can be categorized into three types: predomi-
quent exon E-containing cells, predominant exon E-skipp-
ing cells, and intermediate cells (expressing both exon E-con-
taining and exon E-skipping variants). Hematopoietic progenitor

cells (CD34+CD38− and CD34+CD38+ bone marrow cells) are
predominantly exon E-containing cells, and subsets of thymo-
cytes are either predominantly exon E-containing or interme-
diate type of cells, whereas peripheral T lymphocytes are pre-
dominantly exon E-skipping cells. Hematopoietic cell lines
revealed a similar exon E splicing pattern as their correspond-
ing hematopoietic cells. In general, the expression of the exon E-skipping variant is associated with the maturation of T lymphocytes, whereas the expression of the exon E-containing variant is associated with T lymphocyte immaturity. In this regard, it is interesting to note that peripheral T lymphocytes (CD4+/CD8−) and end stage thymocytes (single CD4+ or single CD8+) have a different exon E-splicing pattern (predominantly exon E-containing type and predominantly exon E-skipping type, respectively). This discrepancy probably reflects a fine difference during the post-thymic maturation process. It has been reported that end stage thymocytes are phenotypically immature and progressively acquire the phenotypic attributes of more mature T cells a few days after release from the thymus (17, 18). This unique phenotype of end stage thymocytes could be used as a potential marker for the identification of recent thymic emigrant (RTE), a newly released population of naive T cells, since this phenotype could still be retained on RTE while disappearing during the maturation process. Recently, CD103 was reported as a feature of the RTE phenotype in humans, which is up-regulated on late CD8+/CD4+/CD3bright thymocytes. This phenotype appears to be retained on a distinct subset of naive CD8+ T cells in the periphery with the expected characteristics of RTE (19). The identification of human RTE is very important for the study of the development of the naive T cell repertoire, the regulation of peripheral T cell homeostasis and naive T cell regeneration after intensive cytotoxic chemotherapy or effective antiretroviral therapy of progressive HIV infection.

Alternative splicing of the chondrolectin gene during the post-thymic maturation process might also be a predictor of certain functional attributes. This is illustrated in the example of the alternative splicing variants of CD45. Variants represent different stages of T cells during maturation, but the corresponding isoforms (CD45RO+ T cells and CD45RO− T cells) display different immunological functions (20, 21). To elucidate the significance of differential exon E splicing during T cell development, it is necessary to understand the function of the full-size isoform CHODLr. We initiated the functional analysis of CHODLr by studying its subcellular localization. Since CHODLr is expressed in various human tissues, it could play a basic role in cells. Therefore, COS1 cells were selected for the transfection and expression of CHODLr. The fluorescent protein-tagged CHODL (RFP-CHODLr) was used for studying the subcellular localization of CHODLr after demonstrating that the protein localization is not influenced by the fluorescent tag. RFP-CHODLr was found in ringlike structures, arrayed perinuclearly and/or along the extension of cells. A localization at the plasma membrane was never observed. Fluorescent protein-tagged CHODL2fK (GFP-CHODL2fK) appears in granule-like structures, scattered throughout the whole cytosol. The shift in localization from ring-like structures of RFP-CHODLr to granule-like structures of GFP-CHODL2fK suggests that the transmembrane region (exon E) is crucial for its anchoring in intracellular membranes. Using double label immunofluorescence with several organelle markers, we demonstrated that RFP-CHODLr colocalizes with rBet1, a transmembrane protein that mediates protein transport between the ER and the Golgi apparatus. This is clearly reminiscent of calnexin, calreticulin, and VIP36. Calnexin (membrane-bound) and calreticulin (soluble) are homologous ER lectins that bind transiently to virtually all newly synthesized glycoproteins. They promote the correct protein folding and provide quality control by preventing incompletely folded glycoproteins from exiting the Golgi complex (7, 8). CHODL2fK has a similar domain structure as CHODLr but lacks the transmembrane domain and terminates at the C terminus in a QDEL sequence. It is well known that proteins that possess a KDEL at the end of the C terminus reside in the lumen of the ER. Lysine can be replaced by Glutamine without affecting its retention to the ER (22). Although CHODL2fK contains an ER retention signal (QDEL), the GFP-CHODL2fK isoform was not found in the ER. Most likely, this can be explained by the fact that the QDEL-containing tail was extended in frame by the GFP protein. Therefore, it will be necessary to analyze the subcellular localization of CHODL2fK with a free QDEL at its carboxyl-terminal end. The development of specific antibodies for CHODL2fK isoform will be very helpful in defining the precise subcellular localization and permit to assess the protein distribution of the different CHODL isoforms. Furthermore, the identification of the carbohydrate ligand of chondrolectin will provide some insight into the physiological function of the novel chondrolectin isoforms in the development of T cells.

In summary, this study reports for the first time the existence of novel members of the chondrolectin family of C-type lectins associated with T cell maturation. Of all of these isoforms, the transmembrane containing isoform CHODLr is localized at the ER-Golgi apparatus.

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