# Polymerase Chain Reaction Selects a Novel Disintegrin Proteinase from CD40-Activated Germinal Center Dendritic Cells

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## Summary

To identify genes expressed by a specific subset of dendritic cells found in vivo a polymerase chain reaction–based cDNA subtraction technique was applied to the recently described germinal center dendritic cells. A novel member of the disintegrin metalloproteinase family was cloned which comprises a not typical zinc-chelating catalytic site most similar to a bacterial metalloproteinase. Dendritic cell precursors or immature dendritic cells express no or low levels of the message. It is induced to high levels upon spontaneous or CD40-dependent maturation and in a mixed lymphocyte reaction. In situ hybridization showed distinct expression of this gene in the germinal center. This, together with the findings that certain disintegrin metalloproteinases regulate the activity of tumor necrosis factor α and that metalloproteinases have also been implicated in FasL processing, suggest that this novel molecule may play an important role in dendritic cell function and their interactions with germinal center T cells.



Dendritic cells (DCs)[1] represent a heterogeneous population of hematopoietic-derived cells that display potent capacity to prime naive T cells and to stimulate memory T cells. DC can be found in an immature form, characterized by a high capacity for antigen capture and processing and a low ability for T cell stimulation (1). Up to now, three types of immature DC have been described: (*a*) Langerhans cells within the epidermis of skin and mucosa, (*b*) marginal DCs within the spleen, and (*c*) $CD4^+CD11c^+$ DCs in blood (human). Upon antigen capture, both Langerhans cells and marginal zone DCs migrate into the T cell areas of regional lymph nodes (through lymph) or of spleen (crossing marginal zone sinuses) where they become interdigitating cells expressing high levels of MHC class II and costimulatory molecules and strongly stimulate antigen-specific naive T cells (2, 3). Blood $CD4^+CD11c^+$ DCs may play an important role in capture and transport of blood antigens into the secondary lymphoid tissues. Recently, $CD4^+CD11c^+$ DCs (GCDCs) have been identified in tonsillar germinal centers (4), suggesting that blood DCs may penetrate into B cell follicles after crossing the high endothelial veinules. Purified GCDCs express low levels of CD40, MHC II, and CTLA-4 ligands, but upregulate these molecules after spontaneous maturation. They induce strong stimulation of $CD4^+$ T cells in vitro and are likely to play a pivotal role in germinal center reactions.

The molecular mechanisms that regulate generation, migration, maturation, or function of different DC subsets in vivo are poorly understood. DCs are hard to isolate and techniques such as producing DC-specific monoclonal antibodies and cloning DC-specific genes by cDNA library subtraction have only been applied to large numbers of DCs generated in vitro from hematopoietic progenitor cells or blood monocytes. Here we describe the application of a PCR-based subtraction technique to three million CD40-activated $CD4^+CD11c^+$ GCDCs isolated from human tonsils with the cloning of a novel member of the disintegrin metalloproteinase family. The gene is strongly expressed in mature DC and in situ can be localized to germinal centers. This novel proteinase may be involved in germinal center reactions, for example, by regulating activities of TNF family members.

## Materials and Methods

*Cell Preparations.* GCDCs were purified from human tonsils according to Grouard et al. (4). After collagenase IV and DNAse digestion of tonsils, cells were centrifuged through a 50% Percoll gradient for 20 min at 400 *g*. $CD3^+$ T cells, $CD19^+$ B cells, $CD14^+$ monocytes (anti CD3[OKT3]), CD19 [4G7], and CD14 [MOP9] mAbs were purified from ascites, our laboratory), and $CD16^+CD56^+$ NK cells (ION16; Immunotech, Marseille, France;

[1]*Abbreviations used in this paper:* DC, dendritic cell; GCDC, germinal center DC; mRNA, messenger RNA; RACE, rapid amplification of cohesive ends; RT-PCR, PCR coupled to reverse transcribed RNA.

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and NKH1; Ortho Diagnostic System, Raritan, NJ) were removed from the collected low density cell population by magnetic beads (anti–mouse Ig-coated Dynabeads M450; Dynal, Oslo, Norway). The remaining cells were stained with mouse anti–CD4-PE-Cy5 (Immunotech), anti–CD11c-PE (Becton Dickinson, Mountain View, CA), anti–CD3-FITC and CD34-FITC (Immunotech), anti–CD20-FITC and anti–CD16-FITC (Becton Dickinson) anti–CD1a-FITC (Ortho Diagnostic System). $CD4^+CD11c^+$ $CD3^-CD20^-CD1a^-$ GCDCs were isolated by cell sorting using FACStar Plus® (Becton Dickinson). In average, a tonsil pair yielded $3 \times 10^5$ cells of 98% homogeneity, a total of 12 tonsil pairs was needed to collect $3 \times 10^6$ cells.

Blood $CD4^+CD11c^+$ DCs were prepared from PBMCs essentially following the procedure for GCDCs. After cell sorting, the purity was >95%. GCDCs and blood DCs were CD40-stimulated for 24 h in complete medium in the presence of 10 μg/ml anti-CD40 antibody G28-5 (provided by Dr. E. Clark). Complete medium is RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 10% (vol/vol) FCS (Flow Laboratories, Irvine, UK), 10 mM Hepes, 2 mM l-glutamine, $5 \times 10^{-2}$ M 2-mercaptoethanol, and 0.08 μg/ml gentamycine (Schering-Plough, Levallois Perret, France).

Stem cell-derived DCs were obtained after 6 and 12 d of culture of CD34 progenitor cells in the presence of TNF-α and GM-CSF (5, 6). Monocyte-derived DCs were produced by incubating purified human monocytes for 5 d in the presence of GM-CSF and IL-4 (7). For CD40 activation, the in vitro–generated DCs were cultured over irradiated murine CD40L-transfected L cells established in this laboratory (8).

Blood mononuclear cells were obtained from human peripheral blood by Ficoll-Hypaque centrifugation. Cells were collected at the interphase and washed twice in complete medium.

T lymphocytes were purified from PBMCs by immunomagnetic depletion using a cocktail of mAbs (IOM2 [CD14], ION16 [CD16], IOT17 [CD35], and ION2 [HLA-DR] from Immunotech; and [CD19] [ascite]). The purity of $CD3^+$ T cells ($CD4^+$ and $CD8^+$) is >95%. Naive T cells were positive selected by anti-CD45RA (ascite, our laboratory).

B lymphocytes were obtained from human tonsils as described (9). T cells were first depleted by rosetting sheep red blood cells and then the residual non–B cells were depleted by T cell–specific antibodies (CD2, CD3, and CD4) followed by anti–mouse IgG-coated magnetic beads (Dynal). The resulting cell population is >98% $CD19^+$ B cells.

Monocytes were purified by $CD14^+$ FACS® sorting after preparation of PBMCs followed by 50% Percoll gradient.

Cell lines (TF-1, MRC5, CHA, U937, JY, and JURKAT) were obtained from American Type Culture Collection (Rockville, MD). Where indicated, cells were activated for 1 and 6 h with 1 μg/ml phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co., St. Louis, MO) and 1 ng/ml ionomycin (Calbiochem Corp., La Jolla, CA) and the time points were pooled.

*RNA Preparation.* Cells were lysed, and total RNA made as described (10). The integrity of the RNA was confirmed by denaturing agarose gel electrophoresis. For GCDC, $3 \times 10^6$ cells were lysed in 0.8 ml lysis buffer (10), and 1/35 volume chloroform/isoamyl alcohol (1:24) was used rather than a 1/20 volume. For tester and driver preparation, $polyA^+$ messenger RNA (mRNA) was selected by oligo $dT_{(25)}$-coupled magnetic beads (Dynabeads Oligo$[dT]_{25}$; Dynal). For U937, three rounds of polyA selection were done before uncoupling $polyA^+$ RNA from the beads. In the case of GCDCs, 100 μl beads (1 mg/ml) was added to the 15 μg total RNA obtained, and after the uncoupling, the remaining total RNA was again selected by three more rounds of beads and finally detached. The quality of U937 $polyA^+$ RNA was verified on a denaturing agarose gel.

*Construction of Subtracted GCDCs cDNA Library.* All of the GCDC mRNA (estimated at 140 ng) was used as tester, and 2 μg U937 mRNA as driver. Complimentary DNA synthesis and subtraction was done in essence following the PCR-select kit (Clontech, Palo Alto, CA; reference 11) using Advantage™ KlenTaq polymerase (Clontech). To compensate for the low amount of tester cDNA, verification of RsaI digestion (step V.B) and the dilution step (step F1) were omitted. The first PCR reaction was done for 28 cycles and the second (nested) PCR for 12 cycles on a thermal cycler (480; Perkin-Elmer Corp., Norwalk, CT). To clone subtracted GCDC cDNA, 10 nested reactions were pooled and resolved on 2% low melting agarose. Aiming for individual bands, 10 gel slices in the 0.7–1.4 kb size range were cut out; the DNA was eluted and cloned either directly or after reamplification into a T/A-vector (pCRII Invitrogen Corp., San Diego, CA). The inserts were sequenced in both directions by automatic sequencing. Comparisons against GenBank and dbest databases as well as protein homology prediction were obtained from the NCBI blast server (http://www.ncbi.nlm.nih.gov/BLAST/).

*PCR.* In the case of the human cell lines and stem cell-derived DCs (Fig. 2 *A*) 50 μg of total RNA was treated with 20 units DNAse (Boehringer Mannheim GmbH, Mannheim, Germany) in the presence of RNAse inhibitor (RNAsin; Promega, Madison, WI) in standard reaction conditions. After phenol/chloroform extraction, the RNA was reverse transcribed using Superscript™II (GIBCO BRL, Gaithersburg, MD) and a $dT_{(12\text{-}18)}$ oligonucleotide, according to instructions (GIBCO BRL). The reaction was stopped, and nucleic acids were ethanol precipitated and resuspended in water. About 20 ng cDNA was used in one PCR reaction. For all other cells, total RNA was prepared from ~100,000 cells, reverse transcribed using Superscript™II (GIBCO BRL) and a random hexaoligonucleotide (pd$[N]_6$; Pharmacia) in a 20 μl reaction. 1 μl was used in a PCR reaction. The 50 μl PCR reaction contained 100 ng primers, 200 μM dNTP (Pharmacia, Uppsala, Sweden), and 0.5 units AmpliTaq™ polymerase (Roche Molecular Systems Inc., Branchburg, NJ) in standard PCR buffer (Roche Molecular Systems) and was subjected to 28 or 35 cycles of denaturing (30 s, 94°C), annealing (30 s, 60°C), and extension (2 min, 72°C) on the Perkin-Elmer thermal cycler 480. Decysin primers U137 and L677 are shown in Fig. 3 *A*, β actin primers were purchased (Stratagene, La Jolla, CA). The Marathon™ kit (Clontech) was used for RACE PCR and performed as recommended by the supplier. The outward primers were 5′-CCCATCAGACCAGATTTCCATACCTACC (upstream) and 5′-CCCATCTTCGGTTGCTGTTATTGAGGCT (downstream), and were used with the Marathon™ recommended cycling program 1. The two distinct PCR products were cloned into the T/A-vector and sequenced.

*Northern and In Situ Hybridization.* $PolyA^+$ RNA blots of human tissues were purchased (Clontech). The original cloned 744-bp fragment was labeled by random priming with [$^{32}$P]dCTP (3,000 Ci/mmol; Amersham Intl., Buckinghamshire, UK; and HiPrime; Boehringer Mannheim GmbH) and unincorporated nucleotides were removed by spin column chromatography (Chromaspin-100; Clontech). Membranes were prehybridized at 65°C in Church solution (0.5 M $NaHPO_4$, pH 7.2, 7% SDS, 0.5 mM EDTA), heat-denatured probe was added, and incubated overnight at 65°C. The membranes were washed under high stringency conditions (0.1 × SSC/0.1% SDS at 65°C) and exposed for 2 wk.

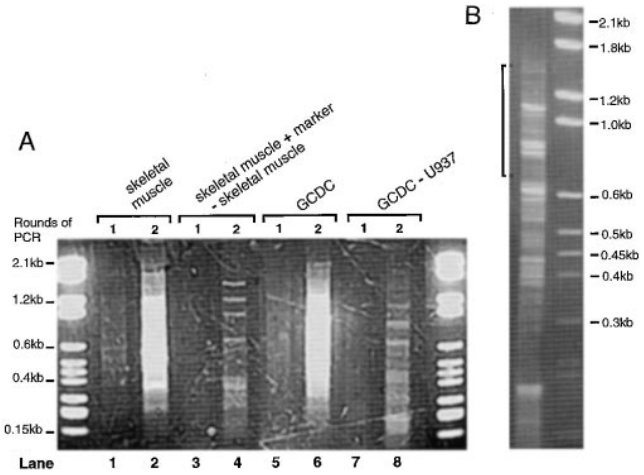


**Figure 1.** Construction of a representative cDNA library from three million CD40-activated GCDCs, subtracted against U937 cDNA. (*A*) Tester cDNA was cut with RsaI, and adapters were ligated and amplified by adapter-specific (round *1*) and adapter-nested (round *2*) primers in the absence (lanes *1, 2* and *5, 6*) or presence (lanes *3, 4* and *7, 8*) of driver cDNA. As positive control skeletal muscle cDNA containing a trace amount of ΦX174 (HaeIII) marker DNA (0.02% of total cDNA) was subtracted against skeletal muscle cDNA (lane *4*). (*B*) 10 times more subtracted GCDC cDNA than in lane *8* was resolved on a long-run low melting agarose gel. DNA within the bracket was recovered and cloned.

In situ hybridization was done as described (12). Sense and antisense $^{35}S$ probes were made by runoff transcription of the 744-bp fragment. 6-μm sections of tonsils were fixed in acetone and 4% paraformaldehyde followed by 0.1 M triethanol amine/0.25% acetic acid. The sections were hybridized overnight, RNase A treated, and exposed for 3 wk. After development, the cells were stained with hematoxyline.

## Results

*Construction of a Subtracted cDNA Library from Three Million CD40-activated GCDCs.* Germinal center DCs were purified from human tonsils (4) and activated by anti-CD40 antibody G28-5 in complete medium for 24 h. From 12 tonsil pairs, a total of three million GCDCs could be obtained, which were estimated to be 98% pure but represented too little material for a conventional subtracted cDNA library without PCR amplification. By modification of the subtractive hybridization technique, termed PCR-select (11), the amount of tester cDNA necessary could be lowered to the 140 ng GCDC mRNA obtained. GCDC cDNA (tester) was cut with RsaI, adapters ligated, and after hybridization in the presence of competitor (driver) cDNA from human monocytic cell line U937, amplified. Thus, the resulting PCR products (Fig. 1 *A*, lane *8*) are restriction fragments of GCDC cDNA absent, or at least rare, in U937. Indeed, individual bands can be seen in lane 8 that are more clearly resolved in *B*. This is obviously different from amplified GCDC cDNA in absence of competitor (lane *6*). As a positive control, subtracting skeletal muscle cDNA containing a trace amount of molecular weight marker against skeletal muscle cDNA only, was able to distinctly expose the added marker DNA (compare lanes *2* and *4*). Given these results, GCDC cDNA fragments from panel *B* in the 0.7–1.4-kb size range were cloned, and 250 clones were sequenced. 30% of the clones contained

**Table 1.** *Genes Highly Expressed in DC of which cDNA Fragments Were Isolated from the GCDC Library*

| Gene | No. of clones of 250 total | Reference |
|---|---|---|
| MHC II | 66 | |
| CD83 | 10 | 44 |
| DC tactin | 14 | |
| human homologue of DEC205 | 1 | 45 |
| Rel B | 6 | 46 |
| IAP-c | 10 | 47 |

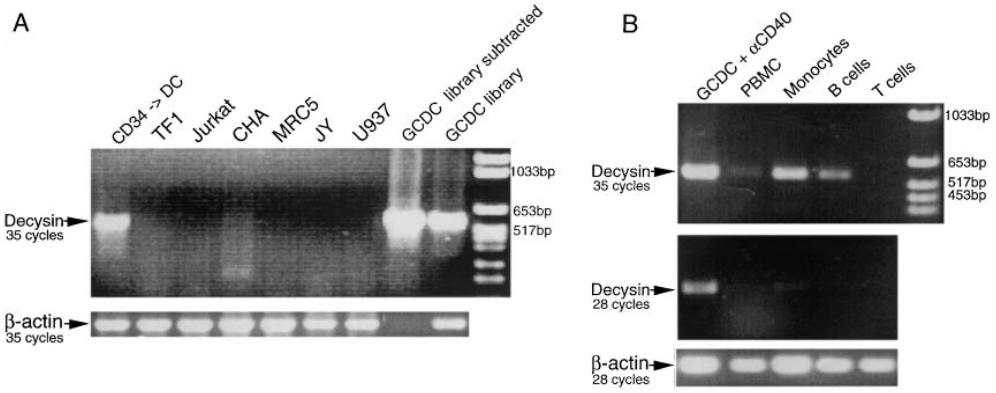


**Figure 2.** Restricted expression profile of decysin by RT-PCR. (*A*) Decysin is not detected in PMA/ionomycin-activated human cell lines TF1 (myeloid precursor cell), JURKAT (T cell), CHA, MRC5 (kidney epithelial and lung fibroblastic cells), and JY (B cell), but is expressed in PMA/ionomycin-activated stem cell–derived DCs. PCR with specific primers to decysin (Fig. 3 *A*) and β actin was performed on reverse transcribed RNA from the cell lines, stem cell–derived DCs harvested at days 6 and 12 of cell culture (6) as well as GCDC library subtracted (Fig. 1, lane *7*) and nonsubtracted (Fig. 1, lane *5*). In the subtracted library, β actin could not be amplified. (*B*) A low level of cDNA is seen in PBMCs, monocytes and B cells. GCDCs were activated for 24 h by αCD40 antibody G28-5 in complete medium. Peripheral blood mononuclear cells, monocytes, and T lymphocytes were obtained from human peripheral blood, and B lymphocytes from human tonsils.

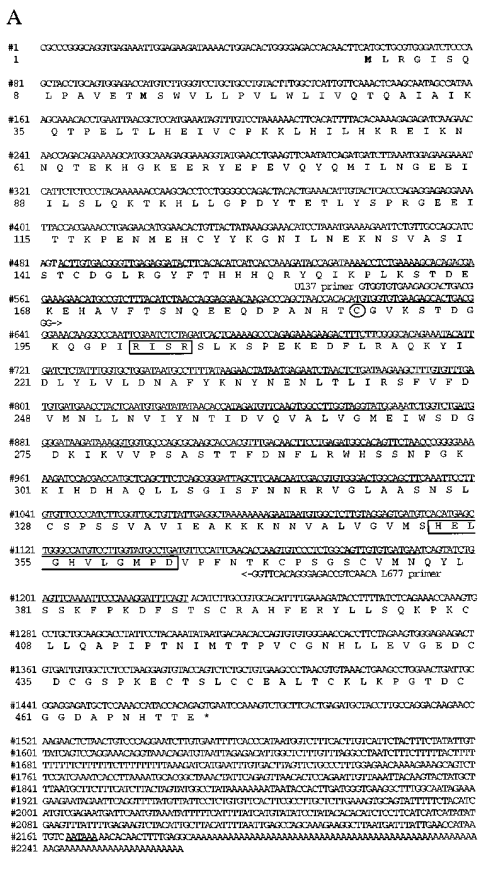

**Figure 3.** A novel member of the disintegrin metalloproteinases. (*A*) Nucleotide and amino acid sequence. The underlined nucleotides represent the original 744-bp RsaI fragment cloned from the GCDC library. The full-length cDNA was cloned from stem cell–derived DCs. The potential polyadenylation site 17 bp upstream of the polyA tail is underlined. Primers used in RT-PCR are shown, which generate a 562-bp product. The first two methionine translation initiation codons are highlighted in bold, and cysteine 187 of the conserved cysteine-switch activation mechanism is circled. The putative furin cleavage site (*RISR*) and the zinc-binding consensus are boxed. These sequence data are available from EMBL/GenBank/DDBJ under the accession number Y13323. (*B*) Comparison of the zinc-binding sites of decysin, ScNP from *Streptomyces caespitosus* (20), and the five subfamilies of zinc endoproteases (38): disintegrin metalloproteinases (snake venom H2 proteinase [39]), matrix metalloproteinases (collagenase [40]), astacin (41), serralysin (42), and thermolysin (43). Decysin and ScNP are the only metalloproteinases in which an aspartic acid (*D*) replaces histidine (*H*) as the third zinc-chelating amino acid. (*C*) Alignment of the disintegrin domains of decysin, mammalian disintegrin metalloproteinases, and the snake disintegrin Ht-e. Decysin displays strong amino acid conservation along the disintegrin domain before prematurely terminating. Other protein sequences continue beyond the last cysteine. EMBL/GenBank/DDBJ accession numbers are given in the figure.

unknown sequences. Among the known, we found a number of cDNA fragments corresponding to genes which are highly expressed in dendritic cells (Table 1). This demonstrates the feasibility of making a high quality subtraction library from a low number of cells.

*Identification of a Gene Predominantly Expressed in DCs.* Among the unknown genes, we retained a clone containing a 744-bp insert because it was not expressed in U937, nor in the following tested human cell lines: TF1 (myeloid precursor cell), JURKAT (T cell), CHA, MRC5 (kidney epithelial and lung fibroblastic cells), and JY (B cell) (Fig. 2 *A*). Yet, it is expressed in CD34 progenitor cell–generated DCs. As control, the cDNA is present in the nonsubtracted GCDC library, and enriched after subtraction. Among freshly isolated cells (*B*), the cDNA could be detected in PBMCs, monocytes, and B cells, but at a lower level compared to CD40-activated GCDCs where 28 PCR cycles were sufficient to generate a clearly visible product. Expression was never observed in T cells. As enough mRNA could not be obtained from GCDC, the expression of this gene in CD34 progenitor cell-generated DCs allowed us to clone the full-length cDNA.

*A Novel Member of the Disintegrin Metalloproteinases.* Several RACE PCR products were sequenced to determine the full-length 2,187-bp nucleotide sequence of the gene which we have named decysin (Fig. 3 *A*). It encodes an open reading frame of 470 amino acids with strong homology to disintegrin metalloproteinases. It has two putative start codons, although the latter is likely to be used more regularly since it more closely resembles a Kozak consensus (13). It is followed by a hydrophobic signal sequence. As a member of the metalloproteinase family, decysin contains a prodomain with a cysteine-switch activation domain at cysteine 187 (14) and a furin cleavage site between residues 200 and 203 (15). Two zinc-chelating histidine (*H*) residues of the zinc binding pocket (*B*) are found at positions 352 and 356 as well as the methionine turn at residue 176 (16, 17). The glutamic acid after the first histidine is strictly conserved in active enzymes. However, distinct from all known mammalian disintegrin or matrix metalloproteinases (18, 19), the third zinc-chelating histidine is replaced by an aspartic acid (residue 362). In the bacterial metalloproteinase ScNP (20), an aspartic acid found at exactly this position was recently identified as the third zinc-chelating amino acid (21; *B*). All mammalian disinte-

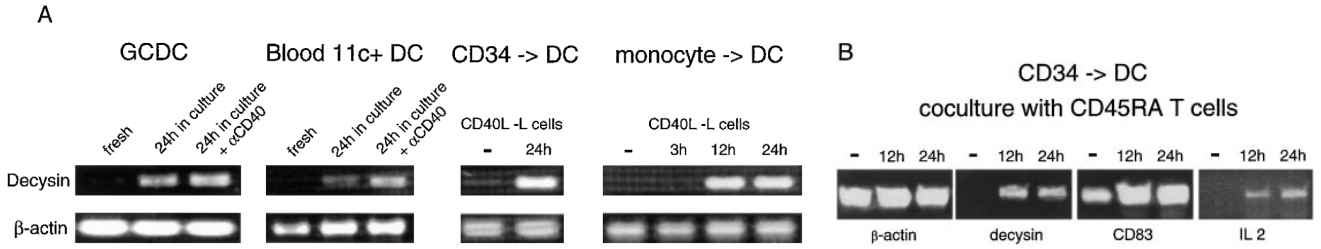


**Figure 4.** Decysin is highly expressed in mature DCs. (*A*) 28 cycle PCR on GCDCs and CD11c$^+$ blood DCs immediately after cell sorting, after 24 h incubation in complete medium and after CD40 stimulation for 24 h in complete medium. Day 12 harvested CD34 stem cell– and monocyte-derived DC were analyzed for decysin before and after CD40 ligation on CD40L-transfected mouse L cells for the indicated time. (*B*) PCR on stem cell–generated DCs before and after coculture with alloreactive total naive T cells for 12 and 24 h. β actin was amplified with 28 cycles, decysin, CD83, and IL-2 with 35 cycles.

grin metalloproteinases share an ~90 amino acid stretch with snake venom disintegrins (22; *C*). Decysin comprises many of these conserved residues, but its open reading frame terminates half way along the consensus. It lacks a transmembrane region that together with the signal peptide, suggests that it is secreted. In summary, by a number of common criteria, the gene codes for a novel member of the disintegrin metalloproteinases with unique features so far unobserved in any other mammalian metalloproteinases.

*Decysin is Induced or Upregulated During DC Maturation.* Since decysin was identified in CD40-stimulated GCDCs, we wondered whether the metalloproteinase might be expressed at high levels in CD40-activated DCs. Indeed, 28 cycle PCR coupled to reverse transcribed RNA (RT-PCR) on freshly isolated GCDCs failed to detect decysin (Fig. 4 *A*). Its expression is induced by spontaneous maturation in culture (4) and increases in response to CD40 activation. Similarly, in another ex vivo isolate, blood CD11c$^+$ DCs do not contain detectable decysin mRNA, but maturation in culture (23) and more importantly CD40 activation result in decysin induction. In vitro generated DCs from CD34 progenitor cells or monocytes rapidly synthesize the message in response to CD40 ligation and a mixed lymphocyte reaction (*B*) results in decysin expression together with CD83. Thus, the novel metalloproteinase represents a DC maturation marker synthesised in response to T cell signals.

*Decysin is Strongly Expressed in Tissues of Chronic Antigen Stimulation.* By Northern blot analysis on different human tissues (Fig. 5), decysin is expressed as a single 2.4-kb message and is highly abundant in the small intestine and appendix. Database searches produced a single partial expressed sequencing tag from pig small intestine (not shown). Expression is also seen in lymph node, mucosal lining of the colon, thymus, spleen, and very weakly in bone marrow. Peripheral blood, ovary, testis, prostate, and fetal liver are negative, as well as other blots containing tissues such as heart, lung, or liver (data not shown).

*In Situ Hybridization Detects Decysin Message in Germinal Centers.* To localize decysin mRNA in human lymph nodes, in situ hybridization was performed (Fig. 6). A tonsil section probed with the antisense RNA strand shows distinct hybridization signals primarily within germinal centers (*A* and *C*) in contrast to the same follicles probed with the sense strand (*B*). The follicle marked by an arrow is shown in higher magnification (*C*). Silver grains are in focalized clusters evenly distributed with the germinal center. This profile is identical to that obtained by anti-CD11c immunostaining of GCDCs (compare *C* and *D*), and confirms that DCs of the germinal center express high levels of decysin.

## Discussion

The study of DCs is hampered by their scarcity in vivo. In this paper, a PCR-based method was used to clone a cell type–specific cDNA from three million CD40-activated germinal center dendritic cells isolated from human tonsils.

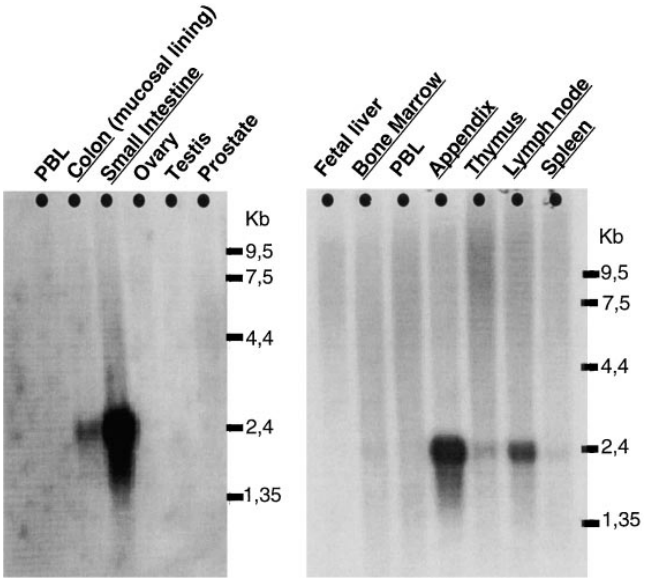


**Figure 5.** Northern analysis. PolyA$^+$ RNA from different human tissues was hybridized with a specific decysin probe. Shown here are multiple tissue blot II (No. 7759-1; Clontech) and immune systems blot (No. 7754-1; Clontech). Not shown are tissue blots No. 7760-1 and No. 7756-1 as they were completely negative. Exposure time was 2 wk.

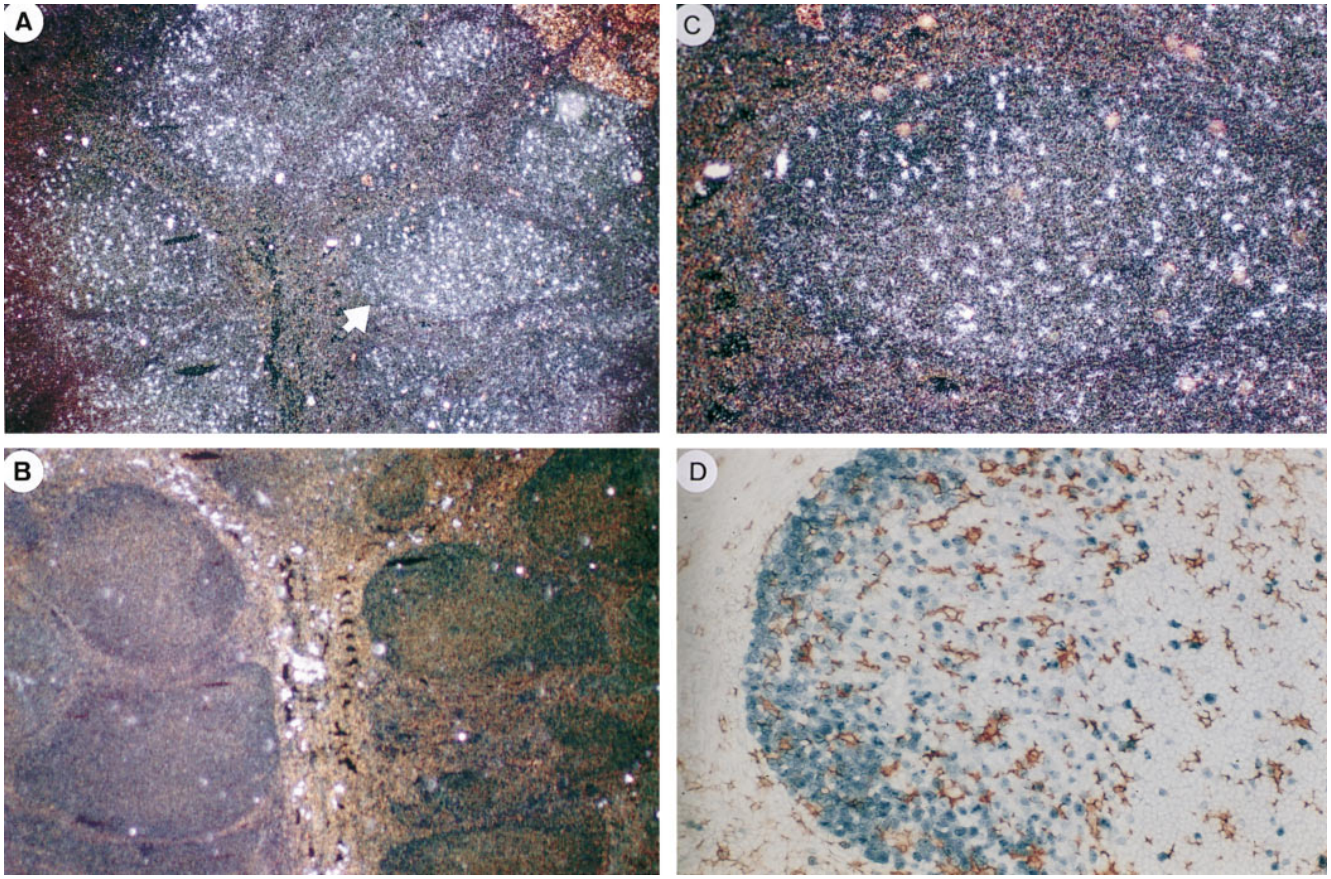


**Figure 6.** Localization of decysin mRNA to germinal centers by in situ hybridization. Human tonsil sections are hybridized with antisense (*A*, *C*) and sense (*B*) decysin $^{35}$S-labeled RNA probes. (*A*) and (*B*) are from serial sections. Original magnification: 40. The germinal center marked by an arrow original magnification: 100 (*C*). The sense probe generates diffuse background hybridization, whereas with the antisense probe, dense clusters of silver grains are seen mainly localized to the follicles. *D* is from reference 4, and shows immunohistological staining of $CD4^+CD11c^+$ GCDCs with anti-CD11c (*red*). In blue are proliferating cells stained by anti-Ki67. Original magnification: 100.

Several lines of evidence indicate the power of this technique. (*a*) Among the 250 sequenced clones, <5% contained common housekeeping genes (β actin could not be amplified from subtracted GCDC cDNA after 35 cycles of PCR [Fig. 2 *A*]). (*b*) A third were unknown genes. (*c*) 107 clones represented genes whose expression is either specific to or highly expressed by mature DCs (CD83, DC tactin, DEC205, MHC class II, Rel-B, IAP-c). On average, we determined that 1 out of 10 clones corresponds to a gene expressed in tester GCDCs and not in driver U937. All these data suggest that the PCR-based subtraction method used here is well applicable to clone unique genes from low number of ex vivo cells, including different DC subsets.

In this subtracted library we identified a novel member of the disintegrin metalloproteinases. It is a large family of mostly membrane-anchored proteases with conserved disintegrin and cysteine-rich domains and a zinc-chelating pocket, although not all members are active enzymes (19). They encode an adhesive function to cell-surface proteins through the COOH-terminal disintegrin domain and a potential antiadhesive/cleavage function through the zinc-dependent metalloprotease domain. Members of this group encode diverse functions. Fertilin α and β have been implicated in sperm–egg binding and fusion (24), meltrin in muscle cell fusion (25), and *Kuzbanian* in neurogenesis (26). Snake venom disintegrins bind the $\beta_3\alpha_v$ integrin and prevent its interaction with fibrinogen (27). By the use of specific inhibitors, metalloproteases have been implicated in shedding of a number of molecules which play critical roles in the immune system: TGF-α, TNF receptors p60 and p80, FasL, CD30, IL-6 receptor, and L-selectin (28–33). Recently, the enzymes that process TNF-α have been identified as disintegrin metalloproteinases TACE (34, 35) and ADAM-10 (36).

Decysin is a member of the disintegrin metalloproteinase family by the following criteria: it has a hydrophobic leader followed by a prodomain with a cysteine-switch consensus, a mechanism by which a prodomain cysteine ligates the active site zinc, and retains the zymogen in an inactive state. Decysin comprises a zinc-chelating catalytic site with a methionine turn and most of the disintegrin domain. Yet, it is unique in three points. First, the third zinc-chelating residue, a histidine in all other disintegrin and matrix metalloproteinases, is replaced by aspartic acid in analogy to a bac-

terial proteinase. Second, the disintegrin domain is truncated. Third, decysin lacks a transmembrane domain, and is therefore potentially secreted. It will be important to examine whether this gene might represent the first member of a new subclass of mammalian disintegrin metalloproteinases.

RT-PCR distribution analysis showed that decysin is moderately expressed by normal B cells and monocytes, but not by T cells and a wide range of human cell lines, even though they had been treated with PMA/ionomycin which is known to promote processing of TGF-α, TNF-α, TNFR, IL-6R, and L-selectin by metalloproteinases (28–30). Instead, decysin is induced or upregulated after spontaneous or CD40 ligand–promoted maturation of different types of immature DCs in vitro. These include blood $CD4^+CD11c^+$ DCs, tonsillar $CD4^+CD11c^+$ GCDCs, monocyte-derived DCs with GM-CSF, IL-4, and $CD34^+$ progenitor-derived DCs with GM-CSF and TNF. In addition, a reaction with allogenic T cells induces decysin synthesis in stem cell–derived DCs. CD40 ligand failed to upregulate decysin expression of human B cells (data not shown). In situ hybridization confirmed the expression of decysin in germinal centers in a pattern identical to CD11c staining of GCDCs. These data show that decysin is selectively expressed by DC and upregulated by signals of activated T cells. The cloning of decysin together with genes of mature DC markers CD83, DEC205, and DC tactin from CD40-activated GCDCs further support the idea of decysin as a novel DC maturation maker.

The impressive expression of decysin in the small intestine and appendix is likely due to the high abundance of DCs in gut epithelium, the lamina propria, and the Peyers patches which comprise a large population of lymphoid cells (37). The continuous and high antigenic load in these sites may induce chronic DC–T cell interactions. Since the members of disintegrin metalloproteinase such as TACE and ADAM-10 have been shown to process membrane-bound TNF-α precursors, and metalloproteinases have been implicated in processing of other members of the TNF family (Fas-L, CD30, and TNFR), decysin may represent a key molecule in regulating DC–T cell interaction.

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*Note added in proof.* DC tactin is identical to macrophage-derived chemokine recently published (Godiska et al., *J. Exp. Med.* 185:1595–1604.

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