Mannose Receptor Ligand-Positive Cells Express the Metalloprotease Decysin in the B Cell Follicle


*J Immunol* 2001; 167:5052-5060; doi: 10.4049/jimmunol.167.9.5052

http://www.jimmunol.org/content/167/9/5052
Mannose Receptor Ligand-Positive Cells Express the Metalloprotease Decysin in the B Cell Follicle

Chris G. F. Mueller,* Isabelle Cremer,† Pierre E. Paulet,*‡ Shumpet Niida,‡ Norihiko Maeda,‡ Serge Lebeque,§ Wolf H. Fridman,* and Catherine Sautès-Fridman*

Decysin, a gene encoding a disintegrin metalloprotease, is transcribed in human dendritic cells (DC) and germinal centers (GC). We have cloned its murine homologue and show that it is processed by the endoprotease furin before secretion of the catalytic domain. We have defined the cell types that express decysin in mouse spleen in the course of an immune response to T cell-dependent Ags. Like in humans, decysin is transcribed by activated CD11c+ DC that enter the T cell zone from the marginal zone (MZ). In the GC, decysin is expressed by follicular DC and tingible body macrophages. In addition, a MZ cell population expresses decysin and appears to migrate into the B cell follicle. The majority of these follicle-homing cells express the mannose receptor ligand, a marker for the macrophage-like MZ metallophilics. The follicle-homing cells are M-CSF dependent, as they are absent in op/op mice that lack functional M-CSF. This suggests that mannose receptor ligand+ MZ metallophilics differentiate into cells that migrate from the MZ into the B cell follicle. Decysin represents the first marker for this previously unrecognized cell population of the mouse spleen, which may represent a precursor for GCDC and may be specialized in the transport of unprocessed Ag from the MZ into developing GC. The Journal of Immunology, 2001, 167: 5052–5060.
18). However, in response to immunization, MZ metallophilis appear not to migrate into the B cell follicle or to the T cell zone (17).

Studies on tonsils have suggested the presence of T and B cell stimulatory DC in GC (19–21). However, the cellular and microanatomical origin of GCDC is currently unclear. In the mouse, GCDC have not yet been identified. Decysin, a novel disintegrin metalloprotease, has been cloned from a cDNA library made from ex vivo purified human GCDC. In situ hybridization revealed transcription of decysin in human GC, suggesting that decysin is a novel marker for this specialized DC subset (22).

In this study, we have cloned the mouse homologue of decysin and show that in immunized mice it is expressed by CD11c+ DC, FDC, and tingible body macrophages. Strikingly, following immunization, MR-L+ MZ metallophilis express decysin and appear to migrate into the developing GC in the course of the immune response. These MZ-derived decysin+ cells are absent in op/op mice that lack functional M-CSF, supporting the idea that the M-CSF-dependent MZ metallophilis migrate into the follicle. MZ metallophilis may be a precursor for the marine GCDC, and could carry immune complexes from the marginal sinus into the developing GC.

Materials and Methods

Animals

BALB/c mice were purchased from Iffa Credo (L’Arbresle, France) and used at 8–12 wk of age. A total of 4 × 10^7 SRBC (Sanofi Diagnostics Pasteur, Paris, France) was injected i.p. Osteopetrotic op/op and control +/+ mice were used at 3 wk of age and were immunized i.p. with 10 μg alum-precipitated chicken OVA. BALB/c mice were injected s.c. into hind footpads with 20 μg alum-precipitated OVA (grade V; Sigma, St. Louis, MO) and boosted 2 wk after. Polypeptide lymph nodes were removed before secondary immunization (day 0) and 2 days after secondary injection. For each time point, three BALB/c mice and two op/op mice were used, and multiple sections were analyzed with identical results.

Cloning of mouse decysin and plasmid constructions
cDNA was amplified from reverse-transcribed BALB/c mouse spleen mRNA (Clontech, Palo Alto, CA) in a PCR using a primer derived from expressed sequence tag (EST) AA608260 and an upstream primer derived from human decysin. The product was cloned into a T/A vector (pCR2.1; Invitrogen, San Diego, CA) and sequenced. A primer derived from this sequence was used to amplify the 5’ untranslated region using Marathon rapid amplification of cDNA ends (Clontech). The PCR product was directly sequenced from both strands. To obtain the full-length mouse decysin-coding cDNA, the upstream primer (5'-ctcagatcgtcgcctggagtactcggc) and the downstream primer (5'-ctcgagttctgtgatgtggtgg) were used in a PCR reaction with DNA polymerase Turbo (Stratagene, La Jolla, CA) and reverse-transcribed BALB/c spleen mRNA as template. After addition of 3’-adenosine overhangs by Taq polymerase (AmpliTaq; PerkinElmer Roche, Norwalk, CT), the PCR product was cloned into the T/A vector (pCR 2.1; Invitrogen) and sequenced.

The insert was liberated at XhoI sites. introduced on the primers, and subcloned into plasmid pLG (R&D Systems, Minneapolis, MN), which directs expression of a fusion protein with the human IgG1 Fc tail. The XhoI restriction fragment was also subcloned into pCDNA3.1 (Invitrogen) in frame with a carboxyl-terminal myc epitope and a 6X histidine (His) tag. Nucleotide sequence of all constructs was verified by double-stranded sequencing.

In vitro expression and furin convertase cleavage

Myb6/×His-tagged decysin was transcribed with T7 RNA polymerase and translated in rabbit reticulocyte nuclear lysate using the TNT-coupled reticulocyte system (Promega, Madison, WI) in the presence of [35S]methionine (Amersham Pharmacia Biotech, Piscataway, NJ). To purify decysin, lysates were added to 200 μl binding buffer (50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole) containing preequilibrated Ni2+-NTA slurry (Qagen, Chatsworth, CA). After binding for 1 h at room temperature, beads were washed in buffer (50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole), and resuspended in 20 μl furin convertase cleavage buffer (50 mM HEPES, pH 7.5, 1 mM CaCl2, 0.5% Nonidet P-40). Ten microliters were treated with 2 U of purified recombinant human furin (Alexis Biochemicals, San Diego, CA) at 30°C for 1 and 3 h. Proteins were resolved on a SDS-PAGE and visualized by fluorography.

Expression of Fc-tagged recombinant decysin

A total of 3 × 10^8 Cos-7 cells was transiently transfected (lipofectamin; Life Technologies, Rockville, MD) with plasmid pcg decysin. Two days later, cells were preincubated for 5 min in methionine-depleted RPMI medium (ICN, Costa Mesa, CA) in the presence of 10% FCS (Valbiotech, Paris, France) and then labeled for 2 h with 150 μCi/ml pro-mix L-S2 (Amersham Pharmacia Biotech) (70% [35S]methionine, 30% [35S]cysteine). After a chase of 1 h in RPMI 1640 medium (Life Technologies) containing 10% FCS, pulse and chase growth medium were pooled, and protease inhibitors were added. Cells were washed twice in cold PBS and lysed at 4°C in RIPA buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton, 0.5% sodium deoxycholate, 0.1% SDS, aprotinin, PMSF, leupeptin). Cleared cell lysates and growth medium were incubated with protein A-Sepharose CL-4B (Sigma) and then washed with RIPA buffer. Precipitated proteins were resolved by SDS-PAGE and visualized by fluorography.

RNA isolation and RT-PCR analysis

To isolate fresh DC, spleen cells from naive or SRBC-immunized BALB/c mice were isolated by mechanical disruption in cold Mg2+ - and Ca2+-free PBS and loaded on a Ficol gradient (Roche Molecular Biochemicals, Basel, Switzerland). Mononuclear cells were collected and allowed to settle on plastic. After 2 h, nonadherent and loosely adherent cells were detached by washing, incubated with biotinylated anti-B220 mAb RA3-6B2, washed, and incubated with streptavidin microbeads (Miltenyi Biotec, Auburn, CA). After two passages over a MiniMac column (Miltenyi Biotec), B cell-depleted cells were then positively enriched for CD11c+ DC by incubating cells with biotinylated N418 mAbs, streptavidin microbeads, and passage over a MiniMac column (Miltenyi Biotec). RNA was isolated using RNApro (Quantum, Durham, NC) and reverse transcribed using the Ready To Go kit (Amersham). RNA from untreated, LPS-, and TNF-α-stimulated DC line D1 (23) were kindly provided by S. Amigorena (Institut Curie, Paris, France). Decysin mRNA was amplified by primers 5’-gaggaattcactacttctactcagatcgtcgcctggagtactcggc) and 5’-ctcgagttctgtgatgtggtgg) were used in a PCR reaction with DNA polymerase FufTurbo (Stratagene, La Jolla, CA) and reverse-transcribed BALB/c spleen mRNA as template. After addition of 3’-adenosine overhangs by Taq polymerase (AmpliTaq; PerkinElmer Roche, Norwalk, CT), the PCR product was cloned into the T/A vector (pCR 2.1; Invitrogen) and sequenced.

The insert was liberated at XhoI sites, introduced on the primers, and subcloned into plasmid pLG (R&D Systems, Minneapolis, MN), which directs expression of a fusion protein with the human IgG1 Fc tail. The XhoI restriction fragment was also subcloned into pCDNA3.1 (Invitrogen) in frame with a carboxyl-terminal myc epitope and a 6X histidine (His) tag. Nucleotide sequence of all constructs was verified by double-stranded sequencing.

Immunohistochemistry

Acetone-fixed spleen sections were incubated with anti-decysin pAb (0.5 μg/ml) overnight at 4°C in PBS/2% goat serum, or with the following Ab for 1 h at room temperature in PBS/2% goat serum: N418 (5 μg/ml), Moma-1 (1/25 dilution), biotinylated RA3-6B2 (2.5 μg/ml), biotinylated peanut lectin (PNA; 2.5 μg/ml), FDC-M2 (1/200 dilution), FA11 (1/100 dilution). Decysin was revealed by biotinylated goat anti-rabbit Ab (Vector, Burlingame, CA) and alkaline phosphatase (AP)-conjugated avidin-biotin complex (Vector). Color development was in Fast Blue (Vector). For N418, FDC-M2, and FA11, the secondary Ab was HRP-conjugated goat anti-rabbit Ab.

Table I. Abs/probes used in this study

<table>
<thead>
<tr>
<th>Ab/Probe</th>
<th>Murine Ag</th>
<th>Ref./Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>N418</td>
<td>CD11c, DC</td>
<td>6</td>
</tr>
<tr>
<td>Moma-1</td>
<td>MZ metallophilis</td>
<td>BMA Biomedicals</td>
</tr>
<tr>
<td>CR-Fc (probe)</td>
<td>MR-L, MZ metallophilis</td>
<td>17</td>
</tr>
<tr>
<td>Subcapsular sinus</td>
<td>monoclonal antibodies</td>
<td></td>
</tr>
<tr>
<td>RA3-6B2</td>
<td>B220, B cells</td>
<td>BD PharMingen</td>
</tr>
<tr>
<td>PNA</td>
<td>GC B cells</td>
<td>Vector</td>
</tr>
<tr>
<td>FDC-M2</td>
<td>FDC</td>
<td>12</td>
</tr>
<tr>
<td>FA11</td>
<td>Macrolasin/C68, tingible body macrophages</td>
<td>Serotech</td>
</tr>
</tbody>
</table>
anti-rat IgG (Southern Biotechnology Associates, Birmingham, AL) and developed in diaminobenzidine (DAB; DAKO, Carpenteria, CA). For Moma-1, the secondary Ab was AP-conjugated goat anti-rat IgG (Southern Biotechnology Associates), and color development was in Fast Red (Sigma). Biotinylated RA3-6B2 and PNA were revealed by HRP-conjugated streptavidin, and development was in DAB (DAKO).

**In situ hybridization**

The protocol is from reference (24). Mouse decysin-coding cDNA was transcribed in antisense direction by T7 RNA polymerase in presence of digoxigenin UTP. Sense probes were used as control and produced unspecific background staining (not shown). The RNA probe was separated from unincorporated label by gel filtration chromatography (Chroma Spin; Clontech) and used for hybridization without prior alkaline hydrolysis. Acetone-treated cryostat sections were fixed in PBS-buffered 4% paraformaldehyde for 10 min at room temperature. RNases were inactivated in PBS containing 1/1000 vol diethylpyrocarbonate (Sigma) for 30 min at room temperature. Sections were washed at 65°C in 2× SSC for 1 h and in 0.1× SSC for 1 h. Sections were then incubated at room temperature for 2 h with anti-digoxigenin AP-conjugated Fab (Roche Molecular Biochemicals) diluted 1/5000 in buffer A (0.1 M Tris-HCl, pH 7.5/150 mM NaCl) containing 0.5% blocking agent (Roche Molecular Biochemicals). After washing in buffer A, development was done in 50 ml buffer B (0.1 M Tris-HCl, pH 9.5, 0.1 M NaCl, 50 mM MgCl2) containing 175 μl 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and 25 μl nitroblue tetrasodium (NTB; Kirkegaard & Perry, Gaithersburg, MD) for between 1 and 2 days at room temperature. The sections were either counterstained with Fast Nuclear Red (Cerstain; Merck, West Point, PA) or subjected to immunohistochemistry. After reequilibration in PBS, sections were incubated with biotinylated PNA (5 μg/ml) in PBS/2% sheep serum, followed by biotinylated goat anti-human IgG (Southern Biotechnology Associates), and color development was in DAB (DAKO). Alternatively, sections were incubated with 6 μg/ml chimeric probe CR-Fc in PBS/2% sheep serum, followed by biotinylated goat anti-human IgG (Southern Biotechnology Associates) and HRP-conjugated avidin-biotin complex (Vector). Development was with DAB (DAKO). All images were recorded by a 3CCD camera (Hamamatsu, Massy, France) and assembled using Adobe Photoshop (Adobe Systems, Mountain View, CA).

**FIGURE 1.** Amino acid sequence comparison of mouse and human decysin. The peptide leader sequences are underlined. Highlighted in bold type are the furin convertase cleavage sites, the zinc binding sites, and the disintegrin domains. The unique aspartate residue in the zinc binding site is boxed. The mouse decysin sequence has been deposited in EMBL/GenBank/DDBJ under the accession number AJ242912.

**Results**

**Cloning of mouse decysin**

Database searching for EST with significant homology to human decysin identified a nucleotide sequence from mouse colon. The missing open reading frame was cloned from mouse spleen cDNA using a primer derived from the EST sequence and a primer of the 5’ end of human decysin. Comparison of the deduced amino acid sequence with human decysin showed 78% similarity and 65% identity between the two proteins (Fig. 1). Like human decysin, the mouse sequence comprises a peptide leader sequence and a recognition site for furin endopeptidases, which would separate the N-terminal prodomain from the catalytic domain. The catalytic domain contains the zinc binding site and a short disintegrin domain. The zinc binding sites of both sequences comprise aspartate at position 361 (boxed residue), which is a unique feature of decysin among all other mammalian disintegrin metalloproteases that comprise a histidine residue at this position (25).

**Decysin is processed by furin convertase and secreted**

To verify that decysin is cleaved by furin convertase, myc6× His-tagged mouse decysin was synthesized in vitro in the presence of [35S]methionine and purified by Ni2+-chelate affinity chromatography. The labeled protein was mock treated or incubated with recombinant human furin convertase, and cleavage products were analyzed by SDS-PAGE. As shown in Fig. 2A, decysin was cleaved by furin convertase in a time-dependent manner, resulting in two clearly visible protein bands corresponding to the 54-kDa full-length protein and the cleaved 22-kDa catalytic domain. The 22-kDa prodomain was further processed at an internal cleavage site (Fig. 1) and migrated with an apparent molecular mass of 18 kDa. It was poorly visible, as it contains only two labeled methionine residues.

To test whether mouse decysin is secreted after furin processing, it was expressed in Cos-7 cells as an Fc-fusion protein. Following metabolic [35S]methionine labeling, decysin Fc was precipitated using a primer derived from the EST sequence and a primer of the end of human decysin. Comparison of the deduced amino acid sequence with human decysin showed 78% similarity and 65% identity between the two proteins (Fig. 1). Like human decysin, the mouse sequence comprises a peptide leader sequence and a recognition site for furin endopeptidases, which would separate the N-terminal prodomain from the catalytic domain. The catalytic domain contains the zinc binding site and a short disintegrin domain. The zinc binding sites of both sequences comprise aspartate at position 361 (boxed residue), which is a unique feature of decysin among all other mammalian disintegrin metalloproteases that comprise a histidine residue at this position (25).

**Peptide leader**

Mouse

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
<th>Mouse expression</th>
<th>Human expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLEGTFSLTEASNNKLYSLWLV1010V1DATETTPSLKPHREVPRKPLGFQKGLLEN</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
<tr>
<td>MLEGTFSL</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
<tr>
<td>MLEGTFSLTEASNNKLYSLWLV1010V1DATETTPSLKPHREVPRKPLGFQKGLLEN</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
<tr>
<td>MLEGTFSL</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
<tr>
<td>MLEGTFSLTEASNNKLYSLWLV1010V1DATETTPSLKPHREVPRKPLGFQKGLLEN</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
<tr>
<td>MLEGTFSL</td>
<td>LEAF</td>
<td>LEAF</td>
</tr>
</tbody>
</table>

**Results**

**Cloning of mouse decysin**

Database searching for EST with significant homology to human decysin identified a nucleotide sequence from mouse colon. The missing open reading frame was cloned from mouse spleen cDNA using a primer derived from the EST sequence and a primer of the 5’ end of human decysin. Comparison of the deduced amino acid sequence with human decysin showed 78% similarity and 65% identity between the two proteins (Fig. 1). Like human decysin, the mouse sequence comprises a peptide leader sequence and a recognition site for furin endopeptidases, which would separate the N-terminal prodomain from the catalytic domain. The catalytic domain contains the zinc binding site and a short disintegrin domain. The zinc binding sites of both sequences comprise aspartate at position 361 (boxed residue), which is a unique feature of decysin among all other mammalian disintegrin metalloproteases that comprise a histidine residue at this position (25).

**Decysin is processed by furin convertase and secreted**

To verify that decysin is cleaved by furin convertase, myc6× His-tagged mouse decysin was synthesized in vitro in the presence of [35S]methionine and purified by Ni2+-chelate affinity chromatography. The labeled protein was mock treated or incubated with recombinant human furin convertase, and cleavage products were analyzed by SDS-PAGE. As shown in Fig. 2A, decysin was cleaved by furin convertase in a time-dependent manner, resulting in two clearly visible protein bands corresponding to the 54-kDa full-length protein and the cleaved 22-kDa catalytic domain. The 22-kDa prodomain was further processed at an internal cleavage site (Fig. 1) and migrated with an apparent molecular mass of 18 kDa. It was poorly visible, as it contains only two labeled methionine residues.

To test whether mouse decysin is secreted after furin processing, it was expressed in Cos-7 cells as an Fc-fusion protein. Following metabolic [35S]methionine labeling, decysin Fc was precipitated using a primer derived from the EST sequence and a primer of the end of human decysin. Comparison of the deduced amino acid sequence with human decysin showed 78% similarity and 65% identity between the two proteins (Fig. 1). Like human decysin, the mouse sequence comprises a peptide leader sequence and a recognition site for furin endopeptidases, which would separate the N-terminal prodomain from the catalytic domain. The catalytic domain contains the zinc binding site and a short disintegrin domain. The zinc binding sites of both sequences comprise aspartate at position 361 (boxed residue), which is a unique feature of decysin among all other mammalian disintegrin metalloproteases that comprise a histidine residue at this position (25).
Decysin is processed by furin convertase and secreted. A epitope and six histidine residues was synthesized in vitro in the presence of Ni²⁺-chelate affinity and mock treated or incubated with recombinant furin convertase for 1 and 3 h. Unprocessed decysin and proteolytic fragments were resolved by SDS-PAGE and visualized by fluorography. B, Fc-tagged mouse decysin was expressed in Cos-7 cells and metabolically labeled. Cell extracts and culture supernatant were incubated with protein A from cell lysates and culture supernatants and analyzed by SDS-PAGE (Fig. 2B). Cell lysates contained predominantly the unprocessed form, and cell culture supernatants contained almost exclusively the processed catalytic domain. This provides evidence that the decysin catalytic domain is secreted while unprocessed decysin remains intracellular.

A rabbit antiserum was raised against a prodomain peptide and affinity purified. Western blot of myc/His-tagged mouse decysin produced in E. coli showed that the anti-peptide Ab recognizes the unprocessed protein, but not a shorter fragment that migrated with the apparent m.w. expected of the catalytic domain (Fig. 2C). The anti-myc Ab recognized both forms.

Decysin is transcribed by DC of the T cell zone

We wondered whether, like its human homologue, mouse decysin is transcribed by mature DC. The mouse DC line D1 (23) was stimulated by LPS or TNF-α, and the expression of decysin mRNA was tested by RT-PCR. LPS and TNF-α had previously been found to be effective maturation agents for this cell line (23). As shown in Fig. 3A, the message was absent in immature D1 cells, weakly induced by LPS, and strongly induced by TNF-α. Next, CD11c⁺ DC were purified from spleens of naive and immunized mice and tested for the presence of decysin message. Decysin was transcribed in CD11c⁺ DC isolated from naive mice, but its message was up-regulated 1 day after immunization with SRBC (Fig. 3A). The presence of decysin mRNA in DC from naive mice was most likely due to DC activation in the course of cell manipulation. To further analyze the induction of the decysin message in DC as well as to explore its expression profile in situ, we performed in situ hybridization on spleen sections from naive mice and 1 day after immunization with SRBC. As shown in Fig. 3B, in naive spleen, no decysin transcripts were detected in the white pulp, comprising the PALS, that surrounds the central arteriole, and the follicles. Also, the MZ, which delineates the white pulp, and the bridging channel (asterisk) showed no significant signal above background. A weak signal was visible in the red pulp close to the MZ, which may reflect a low level of transcription by red pulp macrophages. The expression profile drastically changed 1 day after immunization (Fig. 3C). Now, cells in the PALS clearly transcribed decysin, which correlated with the immigration of CD11c⁺ DC from the bridging channels (asterisk) into the PALS (Fig. 3D). In contrast, the CD11c⁺ DC residing at the bridging channel expressed little, if any, decysin mRNA. Taken together, these data provide evidence that decysin transcription is induced by immunization, and strongly suggest that decysin is transcribed by mature mouse CD11c⁺ DC.

MR-L⁺ decysin⁺ cells enter the B cell follicle from the MZ

At day 1, a cell population located in the Moma-1⁺ MZ also transcribed decysin (Fig. 3, C and D). To test whether these cells were MZ metallophils, spleen sections 1 day after immunization were double stained for decysin mRNA and MR-L, a marker for MZ metallophils (17). As shown in Fig. 4A, almost all decysin-transcribing cells expressed MR-L and were located at the inner MZ. Few decysin⁺ MR-L⁺ cells were found within the follicle (arrowheads). To further assess whether these cells migrate into the B cell follicle during the immune response, spleen sections 2 days after immunization were stained for decysin mRNA and MR-L (Fig. 4B). Now, cells expressing decysin mRNA and MR-L were clearly seen in the B cell follicle (arrowheads). To analyze whether the decysin⁺ cells migrate toward a developing GC, spleen sections taken from mice 2 and 4 days after immunization were stained for decysin using the anti-decysin anti-peptide pAb and PNA. Two
days after immunization, decysin+ cells were positioned between the MZ and a developing PNA+ GC (between arrowheads) (Fig. 4C). Four days after immunization, the decysin+ cells were no longer seen at the MZ, but were distributed within the GC (arrowheads) (Fig. 4D). Taken together, these data suggest that shortly after immunization, MR-L+ MZ metallophils express decysin and home into the developing GC in the course of the immune response.

In the osteopetrotic op/op mice, MZ metallophils are missing due to the lack of functional M-CSF (26). To verify that the decysin+ cells in the follicle were derived from MZ metallophils, op/op and +/+ littermates were immunized with OVA, and spleen sections were stained for decysin and B220 2 days later. While in the +/+ littermates decysin+ cells were present in the MZ and the outer follicle (Fig. 4E), these cells were absent in op/op mice (Fig. 4F). The remaining decysin+ cells were FDC (see below). Thus, the decysin+ follicle-homing cells are M-CSF dependent, supporting the notion that they are derived from MZ metallophils.

To further substantiate these findings, we investigated whether in the lymph node decysin is transcribed by the MR-L+ subcapsular sinus macrophages (8, 17). Lymph nodes were taken from BALB/c mice before (Fig. 4G) or 2 days after (Fig. 4H) secondary immunization with OVA and stained for decysin mRNA and MR-L. Before immunization, the decysin-transcribing cells were almost exclusively localized in the subcapsular sinus area, and the majority expressed MR-L (Fig. 4G). Occasionally, some decysin mRNA+ cells were seen in the follicle (arrowheads). Two days after immunization, the subcapsular sinus macrophages translocated from the subcapsular sinus toward the center of the lymph node and continued to transcribe decysin (Fig. 4H, arrowheads). Staining of adjacent sections confirmed that the MR-L+ decysin+ had entered the B cell follicle (data not shown). These results extend the observations made in the spleen by showing that also in the draining lymph node, decysin is transcribed by the specialized MR-L+ macrophages that home into the B cell follicle in response to immunization with T cell-dependent Ags.

Decysin is expressed by FDC and tingible body macrophages

Immunized op/op mice showed decysin expression in the follicular center (Fig. 4, E and F). To address the question of whether activated FDC express decysin, spleen sections of BALB/c mice 4–6 days after immunization were analyzed for decysin mRNA and protein expression. In situ hybridization revealed, besides decysin
transcription around the central arteriole, decysin mRNA in distinct areas close to the periphery of the white pulp, where GC were expected (Fig. 5A). Immunohistochemical staining with the anti-decsyn pAb (Fast Blue) and PNA (DAB) in spleen sections 2 days (C) and 4 days (D) after immunization. Decysin™ cells are indicated by arrowheads. E and F, Immunohistochemical staining of decysin using the anti-prodomain pAb (Fast Blue) and B220 (DAB) in spleen sections 2 days after immunization taken from +/? littermates (E) and op/op mice (F). G and H, In situ detection of decysin mRNA (NBT/BCIP) and MR-L (DAB) in BALB/c popliteal lymph node sections, before (G) and 2 days after (H) footpad immunization with OVA. Decysin mRNA and MR-L-coexpressing cells in the follicle are indicated by arrowheads. F, follicle. Original magnifications: A and B, ×150; C–F, ×80; G and H, ×100.

Discussion
Previously, human decysin had been cloned from a cDNA library made from GCDC (22). These DC had been identified in tonsilar GC by virtue of their CD4/CD11c expression (19, 20). In this study, we cloned the murine homologue of decysin (Fig. 1) and showed that, like genuine metalloproteases, it is processed by furin proteases (Fig. 2). However, decysin is distinct from the many other mammalian disintegrin metalloproteases 1) by the replacement of the conserved histidine residue by an aspartate in the catalytic site and 2) by its being secreted.

Human decysin is transcribed by mature DC and in tonsilar GC (22). By the use of in situ mRNA hybridization and immunohistochemistry with a pAb specific for the intracellular prodomain, we have identified the cell types that express decysin during an immune response in the mouse (Table II). In the spleen of nonimmunized mice, decysin is not transcribed in the white pulp or the MZ. However, 1 day after immunization, decysin is clearly transcribed in the PALS (Fig. 3). It had previously been shown that the majority of CD11c™ DC presenting a peptide derived from hen egg lysozyme bound to MHC-II are located in the PALS, whereas DC of the MZ present little peptide (27). This observation suggests that immature DC process Ag at the MZ bridging channels, migrate into the PALS where they mature, and present antigenic peptides to host cells.
to T cells. The decysin transcription pattern correlates with the migration of mature DC from the MZ bridging channels and suggests that decysin is expressed by mature CD11c+ DC. In concordance, the unstimulated DC cell line D1 does not transcribe decysin, but its transcription is up-regulated after maturation with LPS or TNF-α. Maturation of DC is induced by microbial products and by cytokines such as IFN-γ, IL-1, GM-CSF, and TNF-α, which are most likely produced by T cells and macrophages, communicating with the immature DC.

In addition to CD11c+ DC of the PALS in immunized mice, we observed a cell population that expressed decysin in the MZ (Figs. 3 and 4). In the course of the immune response, these MZ decysin+ cells appeared to migrate into the B cell follicle (Fig. 4). The majority of the MZ decysin+ cells carry MR-L, recognized by the CR-Fc probe, which is expressed by MZ metallophils (8, 17, 18). op/op mice, which lack functional M-CSF, are devoid of decysin expression in the MZ, which is in accordance with decysin being expressed by the M-CSF-dependent MZ metallophils (Fig. 4) (26). However, as some MZ-derived decysin+ cells lacked CR-Fc binding, we cannot exclude that other cells, such as MZ macrophages, express decysin. On the other hand, it might be that MR-L is lost in the course of the immune response to release mannosylated Ag bound to the MR-L for uptake by B cells or FDC. In the lymph node, decysin is expressed by MR-L+ subcapsular sinus macrophages, which also migrate into the follicle in response to immunization (Fig. 4) (8, 17).

Berney et al. (8) have shown that MR-L+ subcapsular sinus macrophages expressed low levels of CD11c. Upon immunization, the cells migrate toward the outer B cell follicle and associate with B and T lymphocytes. Purified and re-injected into mice, they were able to prime T cells and induce the production of Ag-specific IgM and IgG1 (8). This suggests that lymph node MR-L+ subcapsular sinus macrophages can differentiate into T and B cell stimulatory DC, features previously described for human interdigitating DC and GCDC (20, 28). This raises the interesting possibility that also splenic MR-L+ decysin+ metallophil differentiate into DC in the outer follicle and GC. However, we could not use decysin as a specific marker for these DC, as tingible body macrophages and...

---

**Table II. Decysin-expressing cells in the mouse spleen or draining lymph node**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Localization Before—After Immunization</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC in the PALS</td>
<td>MZ bridging channel→PALS</td>
<td>CD11c, MR-L</td>
</tr>
<tr>
<td>MZ metallophils</td>
<td>MZ→follicle</td>
<td>MR-L</td>
</tr>
<tr>
<td>Subcapsular macrophages</td>
<td>Lymph node subcapsular sinus→follicle</td>
<td>MR-L</td>
</tr>
<tr>
<td>FDC</td>
<td>Follicle/germinal center</td>
<td>FDC-M2, CD68</td>
</tr>
<tr>
<td>Tingible body macrophages</td>
<td>Follicle/germinal center</td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 5.** Decysin is expressed by FDC and tingible body macrophages. A, In situ detection of decysin mRNA (NBT/BCIP) with counter coloration in Fast Nuclear Red (FNR). B–F, Immunohistochemical staining for decysin using the anti-prodomain pAb (Fast Blue) and for the indicated Ags (DAB). Spleen sections were from mice 4 days (A–D) and 6 days (E and F) after immunization with SRBC. Arrows point to decysin+ cells. CA, central arteriole; F, follicle. Original magnifications: A–C and E, ×40; D, ×125; F, ×150.
FDC also expressed this gene (Fig. 5), thus masking detection of potential murine GCDC.

It has recently been observed in TNF-α- and p55TNF-R-deficient mice that the FDC-M2 mAb stained a cell population in the MZ, and that it is capable of binding immune complexes (29). The authors proposed the existence of a MZ-derived FDC population.

It is possible that the decysin-MZ-derived cells may be identical to these putative FDC precursors.

It has been suggested that cells close to the subcapsular sinus are involved in the transport of immune complexes into the developing GC (16). The cells are likely to be nonphagocytic, as they carry immune complexes on the cell surface. It is probable that these Ag-transporting cells are derived from the MR-L+ decysin+ subcapsular sinus macrophages, as they home into the follicle in response to immunization (Fig. 4) (8, 17). MZ metallophils are capsular sinus macrophages, as they home into the follicle in response to the recognition of MZ metallophils in the developing GC (16). The cells are likely to be nonphagocytic, as they carry immune complexes bound to Fc or complement receptors. It would be interesting to investigate the role of MZ metallophils in Ag transport using op/op mice.

In addition to DC and MZ metallophils, we identified two further cell types that expressed decysin in response to immunization: FDC and tingible body macrophages (Fig. 5). As decysin is not expressed by FDC in naive mice, and few genes are known to be expressed by murine FDC, decysin may represent a useful marker for activated mouse FDC. Our inability to detect mouse GCDC using decysin as a probe could be due to their masking by the FDC network and tingible body macrophages. It may also be that GCDC are restricted to human GC, which are much larger and may require DC to stimulate T and B lymphocytes in GC.

The anti-decysin pAb specific for the prodomain recognizes decysin expressed in the MZ-derived cells, FDC, and tingible body macrophages, but not in DC of the PALS (data not shown). We have verified the specificity of the anti-decysin pAb in mice deficient for decysin, in which the Ab does not produce any cell staining (data not shown). As the prodomain may be rapidly degraded after furin-dependent processing of the zymogen, it is possible that the anti-prodomain Ab only recognizes unprocessed decysin. This may imply that DC process decysin more effectively and/or degrade the prodomain more rapidly than other cells, a likely feature of DC, given their efficient endoprotease and exoprotease machinery involved in Ag processing and presentation. Whether decysin itself plays a role in Ag processing is not known. Like other members of its family (30), it is probable that decysin forms heterodimers with other disintegrin metalloproteins. This would enable it to exert different functions, specific for the different cell types that express decysin. For example, the disintegrin metalloprotease TNF-α-converting enzyme (ADAM 17) not only cleaves TNF-α, but also TGF-β, and may be involved in the processing of L-selectin and amyloprotein precursor (31–33). Interestingly, disintegrin metalloproteases have been shown to cleave complement component C3 (34), which plays a key role in GC formation (35–37). An attractive hypothesis would be that after secretion into the GC environment, decysin cleaves complement component C3 and thus terminates GC reaction.

Despite the key role that GC play in shaping the humoral immune response, exemplified by the susceptibility of hyper IgM patients to infectious diseases (38), GC formation still remains far from being fully understood. Efforts aimed at defining the molecules that regulate cellular interactions in the GC reaction should eventually provide us with a molecular framework necessary to understand the complex cellular cross-talk involved in the formation of the secondary B cell response.

Acknowledgments

We are indebted to L. Martínez-Pomarez for kindly providing us with CR-Fc fusion protein and helpful discussions, M. Kosco-Vilbois for Ab FDC-M2, and A. Regnault and S. Amigorena for D1 RNA. We greatly appreciate critical reading of the manuscript by L. Martínez-Pomarez and Y.-J. Liu.

References


