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*J Immunol* 2001; 167:2172-2178;
doi: 10.4049/jimmunol.167.4.2172
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Human Cathepsin W, a Cysteine Protease Predominantly Expressed in NK Cells, Is Mainly Localized in the Endoplasmic Reticulum

Thomas Wex,2,3,† Frank Bühlting,2,§ Heike Wex,*,‡ Dagmar Günther,*, Ekkehard Weber,* and Dieter Brömme*§

Human cathepsin W (also called lymphopain) is a recently described papain-like cysteine protease of unknown function whose gene expression was found to be restricted to cytotoxic cells. Here we demonstrate that cathepsin W is expressed predominantly in NK cells and, to a lesser extent, in CTLs. Quantitative RT-PCR revealed that NK cells contained ~21 times more cathepsin W transcript than CTLs. The predominant expression of cathepsin W in NK cells was further confirmed by Western blot analysis and immunohistochemistry. IL-2-mediated stimulation of NK cells and CTLs revealed a stronger up-regulation of the cathepsin W gene and protein expression in NK cells (7-fold) than in CTLs (2-fold). Transfection experiments of HeLa cells and biochemical analyses revealed that cathepsin W is exclusively “high mannose-type” glycosylated and is mainly targeted to the endoplasmic reticulum (ER). Interestingly, the ER localization of cathepsin W was also found in NK cells, in which colocalization studies revealed an overlapping staining of cathepsin W and Con A, an ER-specific lectin. Furthermore, subcellular fractionation of cathepsin W-expressing cells confirmed the ER localization and showed that cathepsin W is membrane associated. Based on the results of this study, cathepsin W might represent a putative component of the ER-resident proteolytic machinery. The constitutive expression in NK cells and the stronger up-regulation of cathepsin W by IL-2 in NK cells than CTLs suggest that cathepsin W is not just a marker of cytotoxic cells but is, rather, specifically expressed in NK cells. The Journal of Immunology, 2001, 167: 2172–2178.

Natural killer cells and CD8+ CTLs represent two major cell populations involved in the initial immune response against tumors, viral infections, and allografts (1–3). However, despite the common ability of NK cells and CTLs to lyse target cells, there are differences between both cell types. Whereas NK cells exist as preactivated cytotoxic cells capable of mediating their effector function without depending on presented antigenic peptides (MHC class I independent) (2, 3), CTLs mostly have to be activated by Ag-derived peptides bound to the MHC class I complex, and therefore, they are considered to act MHC class I dependently (3, 4). In general, two different cytotoxic pathways are known (reviewed in Ref. 5). The first, the perforin-independent lytic pathway, occurs in Ca2+-free conditions and was found predominantly in CTLs. This way is triggered through cell surface receptors such as Fas/Apo-1 (CD95) and TNFR (CD97) (6, 7). The second, the secretory mechanism, is perforin dependent and was found in both NK cells and CTLs. Here, binding of a target cell by a CTL or NK cell stimulates a Ca2+-dependent degranulation process in the effector cell that leads to a release of a “death cocktail,” causing the subsequent lysis of target cells (5, 8).

The most prominent components of this death mixture are perforin and a family of serine proteases termed granzymes, which are both known to be specifically expressed in CTLs and NK cells (9). Besides the granzymes, there are also cysteine proteases involved in the secretory pathway. Dipeptidyl peptidase I (synonymous with cathepsin C) was shown to be the only enzyme capable of processing progranzymes into their active forms by removing a Glu–Glu dipeptide from the proforms (10, 11). Furthermore, the members of the caspase family mediate the downstream events that finally lead to the death of the target cell (12). Interestingly, some other cysteine protease-related proteins such as the CTL Ag 2-β (13), the cysteine protease inhibitor leukocystatin (14), and cathepsin W (15) were found to be predominantly expressed in cytotoxic cells, suggesting specific functions of these proteins. The potential involvement of other cysteine proteases in cytotoxic processes is further supported by data showing the blocking of TCR-induced death of T cells by papain-like-specific protease inhibitors (16).

Recently, the cDNA encoding a novel papain-like cysteine protease, designated cathepsin W (also referred to as lymphopain), was cloned and further characterized by our group and others (15, 17). Protein sequence alignments showed that this enzyme is a member of the papain superfamily, and that all major structural features such as the positions of the amino acids forming the catalytic triad, the putative cleavage sites for the signal sequence, and the propeptide are in accordance with other members of this protease family (15). The in vivo gene expression of cathepsin W studied by Northern blot analyses revealed a high abundance of the corresponding mRNA in peripheral cytotoxic cells and related cell.

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0022-1767/01/$02.00

Received for publication February 29, 2000. Accepted for publication June 7, 2001.

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1 This work was supported by a fellowship (Wo/2170/2-1) and in part by the SFB 387 (project B7) of the Deutsche Forschungsgemeinschaft (Germany).

2 T.W. and F.B. contributed equally to this work.

3 Address correspondence and reprint requests to Dr. Thomas Wex, Department of Gastroenterology, Hepatology, and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany. E-mail address: thomas.wex@medizin.uni-magdeburg.de or Dr. Dieter Brömme, Department of Human Genetics, Mount Sinai School of Medicine, Box 1498, Fifth Avenue at 100th Street, New York, NY 10029. E-mail address: Dieter.Bromme@msm.edu
lines only, whereas other hematopoietic cells such as CD4 T cells, B cells (CD19), and monocytes (CD14) did not contain significant amounts of cathepsin W mRNA (15, 17).

In this study, we describe the cellular and subcellular localization and regulation of endogenous cathepsin W, which is predominantly expressed in NK cells and was found to be up-regulated by IL-2. Furthermore, we provided evidence that endogenous cathepsin W might be mainly localized in the endoplasmic reticulum (ER), a novel compartment for papain-like proteases.

Materials and Methods

Expression of recombinant cathepsin W

Previously cloned cathepsin W cDNA (15) was used as a template for PCR amplifications with Pfu polymerase (Stratagene, La Jolla, CA) using the following primers that contained either a HindIII or XmaI restriction site for cloning (primer 1, 5'-AAA AAG CTT ACC GGC ATG GCA GGA GCC CAC-3'; and primer 2, 5'-AAA TCT AGA TCA GGG AGG AGA GGA GAC TCG-3'). In addition, a similar PCR product was obtained using primers 1 and 3; the latter was extended by the T7 epitope (primer 3, 5'-AAA TCT AGA GGC TAT CCC ATC TGC TGT CCT CCA GTC-3'). Both PCR products were subcloned into the expression vector pcDNA 3.1 (Invitrogen, Carlsbad, CA). The insert and flanking regions of the vector were sequenced in both directions using an Applied Biosystems Model 377 Automated Sequencer (Applied Biosystems, Foster City, CA).

Preparation of PBMC and purification of CTLs and NK cells

Human PBMC were isolated from healthy donors by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation. Subpopulations of PBMC were enriched by anti-CD4, -8, -19, -56, -34, or -54 microbeads, respectively. NK cells were isolated from PBMC by a two-step protocol. T cells were isolated from PBMC by anti-CD4 microbeads, and this cell population was subsequently used to isolate Th cells or CTLs by anti-CD8 microbeads, respectively. All preparations were done according to the manufacturer's protocol (Miltenyi Biotec). The purity of cell populations was analyzed by flow cytometry and was, in general, between 95 and 99% (relative cell purity), which was detected using 105 Trl X-100 (three times for 20 min), the cells were treated with anti-mouse IgG-FITC or anti-mouse IgG-tetramethylrhodamine isothiocyanate (TRITC), which were both obtained from Sigma (St. Louis, MO) and used as recommended by the manufacturer. After five washing steps with PBS/T containing 0.05% Triton X-100, cells were mounted using Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL) and viewed with a fluorescence microscope (Eclipse E-800; Nikon, Melville, NY).

Electrophoresis and Western blot analysis

In general, SDS-PAGE was conducted by following the method of Laemmlı (20). Briefly, samples were heated in reducing sample buffer at 95°C for 5 min and subjected to SDS-PAGE (Fisher Scientific). Electrophoresis was performed at 50 V for 30 min and 120 V for another 1.5 h using the Mini Gel system (Bio-Rad, Hercules, CA). Prestained protein marker (Life Technologies) was used as the molecular mass standard. Proteins were electroblotted onto nitrocellulose membrane (Fisher Scientific) at 35 V for 3 h (4°C) using the buffer system that contained 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol. Membranes were blocked in 7.5% nonfat milk in TBST containing 0.05% Triton X-100 (TBST/T) for 1 h. After rinsing the membrane twice with TBST/T, the membrane was incubated with the primary Ab for 1–2 h at room temperature or at 4°C overnight, washed four times with TBST/T for 15 min, and incubated with the corresponding secondary Ab (anti-mouse- or anti-rabbit-HRP conjugate; Fisher Scientific) at room temperature for 1 h. After four washing steps, the IgG was detected with the Lumi-Light chemiluminescence detection system (Roche Molecular Biochemicals, Indianapolis, IN) as recommended by the manufacturer.

Quantitative RT-PCR analysis

Total cellular RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany); 500 ng-1 µg total RNA were randomly primed and reverse transcribed by 100 U Moloney murine leukemia virus reverse transcriptase (Amersham Pharmacia Biotech) using the standard protocol recommended by the manufacturer. An aliquot of the cDNA mixture was applied to enzymatic amplifications that were performed using primers 5 and 6, 5'-GCG GCC TGG CCA GTG AAA AG-3'; and primer 6, 5'-GTG CAG CCG GAA ATA GCT CT-3'). Both PCR mixture contained 0.2 mM dNTP (Roche Diagnostics, Mannheim, Germany), 0.5 µM of each primer, reaction buffer with BSA containing 3 mM MgCl2, SbyrGreen (both obtained from Idaho Technology, Salt Lake City, UT), and 1 U Goldstar DNA Polymerase (Eurogentec, Brussels, Belgium). The amplification and online monitoring of the PCR was performed using the LightCycler (Idaho Technology), and an aliquot was analyzed by agarose gel electrophoresis. The initial amount of template was calculated using the LightCycler software. For calibration of the PCR, the PCR product was cloned into the PCRII-plasmid (Invitrogen) that was transformed and expanded in Escherichia coli using standard procedures and later used as standard.

Subcellular fractionation of cell lysates

Cells were resuspended in 250 mM sucrose-20 mM HEPES (pH 7.2) and homogenized in an ice bath using a syringe with a 27-gauge needle. After centrifugation (50,000 × g at 4°C), 5 ml postnuclear supernatant was mixed with 3 ml 80% Percoll and subjected to ultracentrifugation (68,000 × g) for 2 h at 4°C, fixed rotor). Finally, 18–20 fractions were collected from the top (light density) to the bottom (high density). An aliquot was removed for the analysis of the lysosomal marker enzyme (cathepsin B-like activity), which was detected using 10 µM Z-Arg-Arg-methyl ester substrate (Sigma) in acetate buffer (pH 5.5) as previously described (21). The fractions were stored at −80°C for subsequent Western analysis of cathepsin W and the 58K-9 Golgi protein (G2404; Sigma).

Results and Discussion

Characterization of anti-cathepsin W Abs

A fusion protein containing prepro-cathepsin W and an N-terminal located vector-encoded T7 epitope (42 kDa) was expressed in E. coli, gel-purified, and used to generate polyclonal and mAbs. Polyclonal anti-cathepsin W Abs (R-W47/48) were further purified by affinity chromatography using nitrocellulose-blotted recombinant E.coli-expressed cathepsin W as bait. As shown in Fig. 1B, the E.coli-derived T7-cathepsin W fusion protein was recognized by either the mAb against the T7 epitope (mAb-T7) and the purified

4 Abbreviations used in this paper: ER, endoplasmic reticulum; PBS/T, PBS containing 0.1% Tween 20 (pH 7.4); TRITC, tetramethylrhodamine isothiocyanate.

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anti-cathepsin W Abs (R-W47/48), respectively, whereas the serum did not show specific signals. Both polyclonal and monoclonal anti-cathepsin W Abs were analyzed regarding their potential cross-reactivity toward other human cathepsins. Only one of nine monoclonal supernatants tested revealed a cross-reactivity against human cathepsins F, K, S, L, and V, but both of them strongly recognized recombinant cathepsin W (Fig. 1A). To analyze the specificity of the anti-cathepsin W Abs in human cells, cathepsin W expression was analyzed in cathepsin W cDNA-transfected HeLa cells. As shown in Fig. 1C, the Ab R-W47/48 detected a major double band at 50–55 kDa as well as a weak minor band at 42 kDa in HeLa cells transfected with the cathepsin W cDNA. The same results were obtained using monoclonal anti-cathepsin W Abs (data not shown). When stably transfected HeLa cells were subjected to immunofluorescence using R-W47/48 and M-W401B1, a reticular staining was observed, suggesting the staining of the ER compartment as described previously in transiently transfected HeLa or COS-7 cells (19). All together, the Ab characterization proved that both the polyclonal Ab R-W47/48 and the mAb M-W401B1 are equally suitable for analyzing cathepsin W expression.

Characterization of endogenous cathepsin W gene and protein expression in NK cells and CTLs and induction of cathepsin W by IL-2

Previous Northern blot analyses revealed a cytotoxic cell-specific gene expression of cathepsin W (15, 17). To characterize the expression of cathepsin W in more detail, we used magnetic separation techniques to obtain leukocyte subpopulations from PBMC samples. RT-PCR analysis confirmed the killer cell-specific cathepsin W gene expression (partially presented in Fig. 2A). Quantitative RT-PCR revealed that NK cells (CD16⁺) contained an average of ~21 times more cathepsin W transcript than CTLs (CD3⁺ CD8⁺ cells), whereas Th cells (CD3⁺ CD4⁺ cells) contained only traces of cathepsin W mRNA (Fig. 2B). Notably, the distribution of cathepsin W transcript and its concentration varied remarkably among different probands (e.g., 3–25 times enrichment of cathepsin W transcript in CD16⁺ vs CD16⁻ cells). To confirm

![FIGURE 1. Characterization of recombinant cathepsin W and anti-cathepsin W Abs. A. Western blot of several human cathepsins detected either by polyclonal R-W47/48 or monoclonal M-W401B1 anti-cathepsin W Ab. Cathepsins F, K, L, S, and V were expressed in Pichia pastoris, and the cathepsin W sample was a cell lysate from HeLa-G12 cells. B. Isopropyl-β-D-thiogalactopyranoside-induced expression of cathepsin W in E. coli BL21. Samples were harvested as indicated, separated on a 10% polyacrylamide gel, and total protein was stained by Coomassie R250. The induced 42-kDa band was identified as the cathepsin W-T7 fusion protein by the anti-T7 mAb or the R-W47/48 Ab, respectively. C. Exclusive asparagine-linked glycosylation of cathepsin W. Left panel. Purified cathepsin W protein was in vitro treated with endoglucanase H for 30 min – 4 h. The deglycosylation was completed after 30 min; no differences were found in aliquots obtained after 60 min, and the same pattern was obtained using a mixture of endoglucanase H and F (data not shown). Right panel. HeLa cells transiently transfected with the cathepsin W cDNA and control cells were incubated for 24 h without (–) and in the presence of 10 μg/ml tunicamycin (T). The cathepsin W was detected by Western blot analysis using the Ab R-W47/48.

![FIGURE 2. Detection of endogenous cathepsin W and quantitative RT-PCR analysis of T cells and NK cells. A. PBMC were either enriched or depleted using anti-CD4, -8, -19, or -56 Abs, respectively, and then subjected to RT-PCR and cytofluorometric analysis. To prove the integrity of the total RNA, the β-actin transcript was detected in all samples. M, Molecular mass marker. B. Quantitative RT-PCR analysis was performed using total RNA from cells as indicated (n = 4–8). On average, NK cells (CD16⁺), CTLs (CD3⁺ CD8⁺) or Th cells (CD3⁺ CD4⁺) contained ~3.2 × 10⁶ copies, 1.2 × 10⁵ copies, or 2.8 × 10⁴ copies per microgram of total RNA, respectively. C. PBMC or subpopulations were isolated as described in Materials and Methods and subjected to Western blot analysis using the Ab R-W47/48 (PBMC) or M-W401B1 (subpopulations). The left panel documents the presence of endogenous cathepsin W in three different PBMC samples, whereas the distribution of cathepsin W in NK cells vs different T cell subpopulations from two probands is presented on the right panel.](http://www.jimmunol.org/DownloadedFrom)
the gene expression studies, the endogenous cathepsin W protein expression was analyzed by Western blot analysis and immunohistochemistry. As shown in Fig. 2C, three independent PBMC samples tested, as well as isolated NK cells and CTLs, contained endogenous cathepsin W that exhibited a protein pattern in SDS-PAGE similar to that of the HeLa-derived transfectants (Fig. 1). Generally, the Western blot analysis confirmed quantitative RT-PCR analyses by showing that NK cells contain more cathepsin W protein than CTLs.

Furthermore, endogenous cathepsin W was detected in immunohistochemical studies. Purified PBMC were simultaneously stained with anti-CD16 and anti-cathepsin W Abs. The double-staining experiments revealed an almost complete overlap between the expression of CD16 and cathepsin W (Fig. 3A–C), indicating that NK cells represent the major population of cathepsin W-expressing cells among human leukocytes.

Although NK cells are known to be preactivated, the cytolytic activity of NK cells can be further enhanced by stimuli such as IL-2 or IL-12 (22). To study the gene regulation of the cathepsin W gene in response to these cytokines, transcript and protein levels of cathepsin W were analyzed in NK cells stimulated either by IL-2 or IL-12 and in CTLs stimulated with IL-2. In NK cells, a 12.8-fold induction of the cathepsin W mRNA level ranging from 3.3-fold to 34-fold (n = 8) was detected in the presence of IL-2 (Fig. 4A). The elevated transcript levels in IL-2-stimulated NK cells were accompanied by the increase of the protein level as well (Fig. 4B). IL-2-stimulated CTLs revealed a 2.1-fold increase of their cathepsin W mRNA content (1.3- to 6.4-fold; n = 3) and slightly elevated protein levels (Fig. 4B). Although Th cells (CD3⁺CD4⁺) showed a similar relative increase of cathepsin W mRNA levels at much lower concentrations, no clear signal was detected in Western blot analysis (Fig. 4B), suggesting that the traces of cathepsin W mRNA in these cells probably results from contaminating CTLs, which might be present in the isolated CD3⁺CD4⁺ cells. The fact that NK cells contained significantly more cathepsin W transcript and protein than CTLs supports the hypothesis that cathepsin W is not just a marker of cytotoxic lymphocytes but is, rather, specifically expressed in NK cells.

Although IL-12 in general is capable of stimulating NK cells in vitro (22), in similar stimulation experiments, it did not alter the expression levels of either the cathepsin W mRNA or the protein (data not shown). The up-regulation of cathepsin W by IL-2 may be mediated by the intermediate affinity IL-2R, whose β and γ subunits are known to be constitutively expressed on NK cells and CTLs. The binding of IL-2 to its receptor triggers downstream events organized in three major pathways, the ras-signaling pathway, the phosphatidylinositol-3 kinase pathway, and the STAT/Jak pathway, that finally result in transcriptional activation and cellular proliferation (23, 24). Cathepsin W is not the only protein in cytotoxic cells whose transcript level is up-regulated by IL-2. Similar results were described for the dipeptidyl peptidase I (25) and the NK lytic-associated molecule (26), which are both known to be critical for the perforin-dependent pathway in the lysis of cells. Obviously, the cathepsin W gene expression is regulated on the transcriptional level, but the underlying mechanisms for its regulation still need to be elucidated. Future studies, particularly promoter studies and those that assess cathepsin W in different subpopulations of cytotoxic cells during the course of the cytotoxic attack, will shed light on the transcriptional regulation of the cathepsin W gene.

**Biochemical characterization of cathepsin W**

Western blot analyses of HeLa-derived transfectants and immune cells revealed an identical protein pattern (Figs. 1, 2, and 4). Interestingly, the molecular mass of the major double band (50–55 kDa) was higher than the 42.1 kDa calculated from the cDNA sequence and detected in the in vitro transcription/translation assay (17). Sequence analyses revealed two potential N-linked glycosylation sites (Asn⁷⁸ and Asn¹⁵⁶) and three putative O-linked glycosylation sites (Ser¹¹³, Ser¹³⁶, and Thr¹²⁴). To study the N-linked glycosylation, transfected HeLa cells were cultivated in the presence of tunicamycin, which prevents any N-linked glycosylation.

**FIGURE 3.** Immunofluorescence localization of endogenous cathepsin W in CD16⁺ cells. Indirect immunofluorescence detection of cathepsin W was performed using the Ab R-W47/48 as described in Materials and Methods. The colocalization of cathepsin W and CD16 in PBMC (C) was shown by using anti-CD16-FITC (A) and anti-cathepsin W detected by anti-rabbit-TRITC (B). The second panel documents the colocalization of cathepsin W detected by anti-rabbit-FITC (D) and the ER (E) detected by rhodamine-succinylated Con A (Vector Laboratories, Burlingame, CA). Separate images were taken in the corresponding channels and later merged using Adobe Photoshop software (Adobe Systems, San Jose, CA) (C and F). The lower panel represents the corresponding negative controls. G, Anti-rabbit-TRITC; H, anti-rabbit-FITC; and I, anti-mouse-FITC.
Furthermore, purified recombinant cathepsin W was treated with either endoglucanase H alone or with a mixture of endoglucanase H and F, which both remove different types of glycosylation from asparagine residues. As presented in Fig. 1C, these experiments revealed a similar shift of 3–4 kDa in the molecular mass of cathepsin W, suggesting the usage of both putative N-linked glycosylation sites and that only high mannose-type glycosylation is present. Because it is known that O-linked glycosylation is less complex (27), the usage of all potential N- and O-linked glycosylation sites might account for just 4–6 kDa, resulting in molecular mass of 46–48 kDa. It is known that some cathepsins exhibit a slightly higher apparent molecular mass in Western blot analysis (E. Weber, Martin Luther University Halle-Wittenberg, Wittenberg, Germany, unpublished observation). Currently, it is not known whether the higher apparent molecular mass of cathepsin W is caused by similar “electrophoretic artifacts” or whether it reflects the presence of additional posttranslational modifications in the protein.

Because the cathepsin W cDNA-encoded protein exhibits all structural features of an active cysteine protease such as the catalytic triad (Cys, His, Asn), recombinant cathepsin W was analyzed regarding its enzymatic activity. Similar to other cathepsins, cathepsin W is translated as a zymogen and requires the proteolytic processing of the polypeptide for gaining enzymatic activity. In general, cathepsin propeptides can be removed by pepsin treatment or autoactivation at acidic pH, and the enzymatic activities of mature cathepsins are determined by the cleavage of peptide-derived substrates (21). But so far, similar studies performed on recombinant cathepsin W have failed to reveal the proteolytic characteristics of cathepsin W. Considering the unique structural features of cathepsin W, it might be possible that the requirements for its enzymatic activity, such as pH and substrate specificity, completely differ from those of other cathepsins, as it was shown recently for cathepsin X (Z). Cathepsin X exhibits a unique carboxypeptidase activity, but only a very weak endopeptidase activity (28).

Subcellular localization of cathepsin W

Taking into consideration the ER-like distribution of cathepsin W in the HeLa-derived transfectants (19), the subcellular localization of cathepsin W was further studied. Keeping in mind that HeLa cells do not usually express cathepsin W, it is notable that other cathepsins, such as cathepsin F, expressed in HeLa cells were localized in vesicles (21). Therefore, it seems unlikely that the ER-like distribution of cathepsin W in transfected HeLa cells is just an artifact. To study the subcellular localization of cathepsin W in immune cells, PBMC were double-stained with Con A and anti-cathepsin W Abs. As shown in Fig. 3, D–F, the majority of cathepsin W protein was found to be colocalized with the ER marker Con A, a plant-derived lectin that preferentially binds ER-resident proteins (5).

FIGURE 4. Induction of human cathepsin W in NK cells and CTL by IL-2. A, A strong increase of the cathepsin W mRNA level was observed in NK cells (12.8-fold; n = 8), whereas CTLs exhibited an increase of 2.1-fold (n = 3) and Th cells of 1.7-fold (n = 3). Cells were incubated with 1,000 U/ml IL-2 for 2 days (left panel). Quantitative RT-PCR analysis was performed using total RNA from NK cells activated with different concentrations of IL-2 for the time periods indicated (right panel). B, The induction of cathepsin W transcript levels (1,000 U/ml, 2 days) was accompanied by elevated protein levels in NK cells and CTLs (Ab M-W401B1), as shown by three independent experiments, whereas no cathepsin W was detected in Th cells (two experiments).
high mannose-type-glycosylated proteins (29). To confirm the ER-like distribution of cathepsin W with an alternative method, subcellular fractionation of cathepsin W-containing cells was performed in a self-generating Percoll gradient. As shown in Fig. 5, cathepsin W was found in the lighter fractions of the gradient of transfected HeLa cells as well as in two PBMC samples, whereas wild-type HeLa cells did not reveal any signal. To assess the quality of the subcellular fractionation, the distribution of Golgi marker 58K-9 (30) and cathepsin B as a lysosomal marker (31) were studied. The 58K-protein was predominantly found in fractions 7–11, whereas the cathepsin B-like activity was mostly identified in the high density fractions (fractions 16–18) of the gradient (Fig. 5). Notably, these fractions did not contain any or contained a very little amount of cathepsin W. To prove the membrane association of cathepsin W, postnuclear supernatants were subjected to ultracentrifugation (100,000 × g for 2 h), and the particulate fraction containing all membrane components and the cytosolic supernatant were recovered. Subsequent Western blot analysis revealed an almost exclusive signal in the particulate fraction, whereas the supernatant contained only traces of cathepsin W (data not shown).

Because ER-resident proteins have to be actively retained from further transportation or retrieved from the Golgi compartment, the cathepsin W cDNA was screened for known protein signals mediating ER retention, but none of these motifs, such as the KDEL motif (32), KDEL-like motifs (33), C-terminal cysteine residues (34, 35), basic dipeptides within KXXK motifs (36), or internal signal sequences (37) were detected. Because, cathepsin W contains two unique motifs, a 21-aa insertion and an 8-aa C-terminal extension, which were not found in any other cathepsin (15), it will be necessary to characterize these unique motifs regarding their ability to mediate targeting of cathepsin W to the ER compartment. Based on the results of the subcellular localization and the almost-exclusive presence of cathepsin W in the particulate fraction, a potential membrane attachment of cathepsin W would be an interesting hypothesis that would explain the higher apparent molecular mass of cathepsin W in the SDS-PAGE and possibly represent the mechanism for the ER retention. Current studies are aimed to determine whether cathepsin W is attached to the membrane and to elucidate the underlying mechanism of its attachment.

Functional implications

Based on the predominant ER localization, cathepsin W might play a functional role in the proteolytic machinery of this compartment. For some years, it has been known that the ER contains a variety of proteolytic enzymes including cysteine proteases (ER-60) either involved in the ER-associated protein degradation or the processing of polypeptides by “limited proteolysis” (38–41). In addition, several studies provided evidence for regulatory functions of ER-resident proteases in the processing of other proteins. The signal peptidase located in the ER membrane is the sole enzyme responsible for cleaving off the signal peptide from all proteins translated into the ER (42). The yeast protease Ste24p and presenilin 1 are other ER-resident proteases involved in the processing of C-terminal CAAX motif and the amyloid precursor protein, respectively (43, 44). Considering the predominant expression of cathepsin W in NK cells and its subcellular localization in terms of a potential involvement in cytotoxic processes, it is tempting to assume that its substrates should be NK cell specific. Studies focused on the enzymatic activity of cathepsin W are currently underway. So far, cathepsin W can be characterized as a cysteine protease that might be a component of the ER-resident proteolytic machinery that is involved in the processing of NK (CTL) cell-specific proteins. The identification of these substrates as well as the full characterization of the intrinsic enzymatic activity of cathepsin W will give more insight into the regulation of ER-resident proteolysis in NK cells and will enable us to develop a functional model for cathepsin W.

Acknowledgments

We are very grateful to Ruth Hilde Hädicke, Marianne Blichmann (Otto-von-Guericke University, Magdeburg, Germany), and Rita Medek (Martin Luther University Halle-Wittenberg) for their excellent technical assistance.

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