Cathepsin S Activity is Detectable in Human Keratinocytes and is Selectively Upregulated upon Stimulation with Interferon-γ

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Keratinocytes are an integral component of the skin immune system and function as nonprofessional antigen-presenting cells in pathophysiologic conditions when they express major histocompatibility complex class II molecules, e.g., in psoriasis. In order to analyze further this function we investigated the activity of cathepsin S in comparison with cathepsins B and L. These enzymes were suggested to be involved in antigen presentation. Specific catalytic activities of these cathepsins were determined fluorometrically by hydrolysis of a synthetic substrate (Z-Phe-Arg-7-amido-4-methylcoumarin) in subcellular fractions of human keratinocytes. It was found that the human keratinocyte cell line HaCaT exhibits activities of all three cathepsins investigated. Endosomal/lysosomal compartments show highest cathepsin activities. Normal human keratinocytes in primary culture show a comparable pattern of cathepsin activities. In contrast to this, in syngeneic Epstein–Barr virus-transformed B cells the level of cathepsin B activity was found to be 10% of that in the corresponding keratinocytes, whereas the activities for cathepsins L and S were in a similar range. Interferon-γ stimulation of primary keratinocytes and HaCaT cells resulted in a selective upregulation of the cathepsin S activity, the extent of which was very similar. The mechanism of this upregulation was demonstrated as induction at the mRNA and protein levels. This report documents that cathepsin S in human keratinocytes is selectively upregulated, in parallel to major histocompatibility complex class II molecules, in response to a pro-inflammatory cytokine. Our observations support the concept of keratinocytes functioning as nonprofessional antigen-presenting cells in states of inflammation. ***Key words: antigen presentation/antigen processing/cathepsins B, L, S/ psoriasis. J Invest Dermatol 119:44–49, 2002***

The task of immune surveillance and immune responses as an integral part of the barrier function of the skin is mirrored by the complex composition of the so-called "skin immune system" (Bos and Kapsenberg, 1986). In order to mount a specific immune response this system needs to generate antigenic peptides and to present them in a fashion allowing stimulation of lymphocytes, a function usually performed by professional antigen-presenting cells, e.g., Langerhans cells, which are characterized by their major histocompatibility complex (MHC) class II positive phenotype (Germain, 1994). Cytokine stimulation *in vitro* or the state of inflammation *in vivo*, however, results in the induction of MHC class II expression on a variety of endothelial and epithelial cells, including keratinocytes (Gottlieb *et al*, 1986). This phenomenon for which interferon-(IFN)γ seems to play a key part (Kerr *et al*, 1990; Albanesi *et al*, 1998) turns keratinocytes into nonprofessional antigen-presenting cells (Nickoloff and Turka, 1994).

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Reprint requests to: Dr. Hubert Kalbacher, Medical and Natural Sciences Research Center, Ob dem Himmelreich 7, D-72074 Tübingen, Germany. Email: kalbacher@uni-tuebingen.de Abbreviation: NAG, *N*-acetyl-β-D-glucosaminidase.

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Besides MHC class II expression for antigen presentation the ability for peptide processing is relevant for a cell to fulfill the function of an antigen-presenting cell effectively. Generation of antigenic peptides, as intracellular protein degradation in general, involves a family of endosomal/lysosomal proteases called cathepsins. Cathepsins can initiate antigen processing by generating larger fragments containing antigenic epitopes, the respective fragments are subsequently trimmed to their final size by exopeptidases (Fineschi and Miller, 1997). For the generation of T helper cell epitopes from ovalbumin and myoglobin involvement of cathepsins B, D, and L has been demonstrated (Takahashi *et al*, 1989; Van Noort and van der Drift, 1989; Diment, 1990). Additionally, cathepsin S may be of particular relevance in the context of antigen processing as it is known to degrade the invariant chain to class II associated invariant chain peptide, an important step for the correct loading of MHC class II molecules with antigenic peptides and subsequent surface expression of this complex (Riese *et al*, 1996).

To analyze further the role of keratinocytes as nonprofessional antigen-presenting cells of the skin immune system the activity of cathepsins S was determined in comparison with the activities of cathepsins B and L presumably relevant for antigen presentation in human keratinocytes. In addition, these enzyme activities were compared with those of the professional antigen-presenting B cells. Qualitative similarities were observed in the pattern of cathepsin

activities in syngeneic keratinocytes and B cells and selective upregulation of cathepsin S activity in IFN-γ-stimulated keratinocytes.

MATERIALS AND METHODS

Cells The HaCaT line of spontaneously immortalized human keratinocytes (Boukamp *et al*, 1988) was a kind gift from Prof. Fusenig (German Cancer Research Center, Heidelberg) and was maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, penicillin 100 IU per ml, 100 μg streptomycin per ml or 130 μg gentamycin per ml (Gibco Life Technologies, Paisley, U.K.). Normal human keratinocytes were derived from the foreskin of one patient (or collected from four patients) who underwent circumcision and cultured according to the method of Rheinwald and Green (1975) in keratinocyte growth medium (Promo Cell, Heidelberg, Germany) supplemented with bovine pituitary extract (Gibco Life Technologies) as recommended, 100 IU penicillin per ml and 100 μg streptomycin per ml or MCDB 153 medium (Sigma, Deisenhofen, Germany) and supplemented according to Meier *et al*, 2000. HaCaT cells and normal keratinocytes were cultured in a humidified atmosphere containing 5% CO_2 at 37°C. Epstein–Barr virus (EBV)-transformed B cells were established from peripheral mononuclear cells of the same individual, who underwent circumcision, and cultured in RPMI 1640 with 10% heat-inactivated fetal bovine serum in roller bottles at 37°C. HaCaT cells as well as keratinocytes were stimulated for 72 h with recombinant human IFN-γ at a concentration of 1000 units per ml (Thomae, Biberach and Strathmann Biotech GmbH, Hamburg, Germany).

Fluorescence-activated cell sorter analyses To characterize further the effects of IFN-γ on the antigen presenting abilities of keratinocytes expression of HLA class I and class II molecules as well as invariant chain was investigated by means of fluorescence-activated cell sorter analyses (FACScan, Becton-Dickinson, Mountain View, CA). The following mouse anti-human monoclonal antibodies were used: W6/32 detecting HLA-A, -B, and -C (ATCC, Manassas, VA); L243 detecting HLA-DR (ATCC, Manassas, VA); FA for HLA-DP (ATCC, Manassas, VA); TÜ22 for HLA-DQ, and $SD_3$253.74 for invariant chain. The antibodies TÜ22 and $SD_3$253.74 were kindly provided to us by Prof. Dr Claudia A. Müller/Department of Medicine of the University of Tübingen.

Cell fractionation As the cellular cytosol contains strong cathepsin inhibitors (Abrahamson, 1994), the enzyme activities were determined in whole cell lysates as well as in different cellular compartments. Cell fractionation was performed by differential centrifugation as described by Schröter *et al* (1999). Briefly, the different cell preparations (at least 20–40 millions cells each) were collected, washed, and homogenized in an Elvehjem-Potter containing a homogenization buffer (250 mM sucrose, 10 mM Tris, pH 7.2), followed by centrifugation (Beckman TL 100 ultracentrifuge). The following fractions were obtained: debris (4000 × *g* for 2 min), mitochondria and endosomes/lysosomes (100,000 × *g* for 2 min), microsomes (400,000 × *g* for 12 min), and cytosol (supernatant). Lysosomes were separated from endosomes by hypotonic lysis using 2.5 times of the pellet volume for keratinocytes and five times of the pellet volume in the case of B cells, followed by repeated centrifugation with 100,000 × *g* for 4 min. In addition, a crude organelle fraction was prepared by omitting the second centrifugation step (100,000 × *g* for 2 min). The pellet obtained after centrifugation at 400,000 × *g* for 12 min consists of mitochondria, endosomes, lysosomes, and microsomes.

***N*-acetyl-β-D-glucosaminidase (NAG) assay** As NAG is a good marker for endosomal/lysosomal compartments, determination of NAG activity was used to control the quality of the cell fractionation using a method described by Schmid *et al* (1993): Aliquots are added to 100 μl of 0.1 M citrate buffer (pH 5.0; 2–50 ng protein per μl) containing 0.8 mM 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide (Sigma, Deisenhofen, Germany) and 0.1–1% Triton X100. Fluorescence was measured every 8 min at 37°C using a fluorescence reader (Tecan SpectraFluor, Crailsheim, Germany; excitation wavelength 360 nm, emission wavelength 465 nm). NAG activity was determined by linear regression using a minimum of four measurement points. 4-methylumbelliferone was used for calibration.

Determination of cathepsin activities Specific catalytic activities of cathepsins B, L, and S were determined fluorometrically by hydrolysis of the synthetic substrate Z-Phe-Arg-7-amido-4-methylcoumarin (Bachem, Bubendorf, Switzerland) (Morita *et al*, 1977) according to Schmid *et al* (1997) with the following variations. For total cathepsin activity (B, L, and S), 100 μl of 0.1 M citrate buffer pH 5.0 containing 4 mM dithiothreitol, 4 mM ethylenediamine tetraacetic acid, and 6 μM aprotinin were used; an aliquot probe (2–50 ng protein per μl) was added and the reaction was started with substrate solution (500 μM). The liberated fluorescence was measured at 37°C every 10 min with a fluorescence reader (Tecan SpectraFluor; excitation wavelength 360 nm, emission wavelength 465 nm). Activities were determined by linear regression using a minimum of four measurement points. Addition of the specific inhibitor CA074 (10 μM; Bachem, Bubendorf, Switzerland) (Towatari *et al* 1991) to the same reagent yielded cathepsin B activity (inhibited activity). The remaining activity was confirmed to be cathepsin L activity by inhibition with 0.5 μM Z-Phe-Phediazomethylketone in a separate experiment. For determination of cathepsin S activity an aliquot probe was preincubated with 0.1 M phosphate buffer (pH 7.5) containing 4 mM dithiothreitol, 4 mM ethylenediamine tetraacetic acid, 6 μM aprotinin at 37°C for 1 h (0.1–1.2 μg protein per μl). By this procedure inactivation of cathepsins B and L was achieved (Kirschke *et al*, 1989; Schmid *et al*, 1997). After adding substrate solution (500 μM) incubation for 60 min yielded cathepsin S activity, which was inhibited by 5 nM morpholineurea-leucinyl-homophenalanyl-vinylsufone-phenyl (Palmer *et al*, 1995; Riese *et al*, 1996).

Complete inhibition of these enzyme activities by E-64 (10 μM; Boehringer Mannheim, Mannheim, Germany; Hanada *et al*, 1978) confirmed the detection of cysteine proteinase activities. Amido-4-methylcoumarin (Bachem, Heidelberg, Germany) was used for calibration. The activity determinations were performed in duplicate.

Protein concentrations were determined by a modified method of Bradford. Roti-Nanoquant solution (Roth, Karlsruhe, Germany) and bovine serum albumin as standard for quantification were used. Specific enzyme activities were expressed as μmol per min per g of protein.

Reverse transcription–polymerase chain reaction (reverse transcription–PCR) of human cathepsin S (When not explicitly mentioned all materials used were from Peqlab, Erlangen, Germany.) Total RNA was isolated from keratinocytes (controls and stimulated with 1000 U IFN-γ per ml for 24 h) with EZNA total RNA kit. In both cases 1 μg total RNA was mixed with 250 ng oligo(dT) primer (Stratagene, Amsterdam, the Netherlands) in a final volume of 10 μl of diethyl pyro carbonate-treated water, heated to 65°C for 10 min and cooled on ice. Reverse transcription was performed in a final volume of 20 μl of 10 × reverse transcriptase buffer containing dithiothreitol (2.5 mM), deoxyribonucleoside triphosphate (1.25 mM) and 25 units of avian myeloblastosis virus reverse transcriptase. Samples were incubated for 1 h at 42°C and cooled on ice. The PCR was initiated by pipetting 2–6 μl of the cDNA (100–500 ng), 10 × Taq buffer, deoxyribonucleoside triphosphate (0.2 mM), 50 pmol each of forward and reverse primer, 2 units Taq polymerase and sterile water to a final volume of 50 μl. Amplification was done over 35 cycles with a thermal cycler (Applied Biosystems 9700, Norwalk, CT). Each cycle consisted of following steps: denaturation at 94°C, annealing at 56°C and extension at 72°C for 30 s each. PCR products were identified by 2% agarose gel electrophoresis.

The following sets of oligonucleotide primers were used to amplify cDNA: human cathepsin S 5′ CAGGAAAGCTGGTGACTCTCAG 3′ and 5′ TCTGGGTAAGAGGGAAAGCTAGC 3′ (GenBank accession number XM-002028), human β-actin 5′ TCAGAAGGATTCCTA-TGTGGGC 3′ and 5′ CCATCACGATGCCAGTGGTA 3′ (GenBank accession number NM-001101). The length of cathepsin S DNA segments to be amplified were 500 bp. Primer pairs amplifying a 300 bp segment of the human β-actin gene were chosen as a positive control for the reverse transcription.

Western blotting Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of subcellular keratinocyte fractions (8–15 μg protein) was carried out in a 12% gel followed by electroblotting on to polyvinylidene difluoride membrane (Amersham-Pharmacia, Amersham, Buckinghamshire, U.K.) using a tank transfer unit. After treatment with milk powder the membrane was incubated with polyclonal rabbit antibody against cathepsin S (1:3000) (E. Weber, Halle, Germany) and anti-rabbit antibody conjugated with horseradish peroxidase (Dianova, Hamburg, Germany). Signals were detected by chemiluminescence (ECL-Plus detection kit, Amersham-Pharmacia).

RESULTS

Activities of cathepsins B, L, and S in HaCaT keratinocytes Initially, we attempted to establish the cathepsin activity

pattern in control HaCaT keratinocytes (**Table I**). Cathepsin B activity of the different fractions contributed about 90% of the total cathepsin activities, followed by cathepsin L (about 10%), whereas cathepsin S accounted for less than 0.2% of total cathepsin activity ranging at the detection limits. In analogy to the endosomal/lysosomal marker enzyme NAG, we found highest activity of the three cathepsins in endosomal and especially lysosomal fractions. The ratio of endosomal and lysosomal activities of all four enzymes was very similar with an approximately 5-fold level found in lysosomes.

Activities of cathepsins B, L, and S in normal human keratinocytes in comparison with those in syngeneic B cells As HaCaT keratinocytes are spontaneously immortalized cells we compared their cathepsin activities with those of human keratinocytes in primary culture, obtained from one patient who had undergone circumcision. In these cells the activities of the three cathepsins ranged in the same order of magnitude as in HaCaT cells (**Table II**). Again, the most abundant protease activity was that of cathepsin B followed by cathepsins L and S. In contrast to HaCaT keratinocytes NAG activities of primary keratinocytes were found to be essentially higher than cathepsin B activities.

In order to compare the cathepsin activities found in keratinocytes we determined these enzyme activities in professional antigen-presenting cells. For this purpose, EBV-transformed B cells were derived from peripheral mononuclear cells of the same patient who had undergone circumcision. Interestingly, the B cells exhibited about 10% of cathepsin B activity in whole cell homogenate as well as in subcellular fractions as compared with cathepsin B activities in syngeneic keratinocytes. Cathepsin L activity was close to the level detected in the keratinocytes, with a similar distribution pattern as compared with that of cathepsin B and NAG activities. Again, the lowest activity levels were found for cathepsin S, which were similar to those of keratinocytes. By this way, in the EBV-transformed B cells the percentage of cathespin S activity relative to total activity of cathespins B and L with 0.8% and 1.4% in whole cell homogenate and lysosomal fraction, respectively and 5.1% in the endosomal fraction becomes higher than in the syngeneic keratinocytes. This relatively high endosomal cathepsin S activity is 50% of lysosomal cathepsin S activity, whereas this percentage was essentially lower in primary keratinocytes and HaCaT keratinocytes.

Table I. Lysosomal enzyme activities in subcellular fractions of HaCaT keratinocytes[a]

	Cathepsin B	Cathepsin L	Cathepsin S	NAG
H	32.1 ± 2.6	2.1 ± 0.8	0.03 ± 0.01	19.9 ± 3.5
D	47.7 ± 1.4	1.1 ± 0.1	0.07 ± 0.05	19.5 ± 3.4
E	65.5 ± 7.0	8.3 ± 3.1	0.12 ± 0.09	36.6 ± 4.0
L	348.1 ± 40.2	52.7 ± 1.9	0.60 ± 0.06	149.1 ± 14.6
M	8.4 ± 0.9	0.4 ± 0.4	0.06 ± 0.01	6.4 ± 2.5
C	6.8 ± 0.8	1.0 ± 1.0	0.02 ± 0.02	6.9 ± 1.2

[a]Units per g protein, mean of the values obtained from two experiments with at least two determinations each. H, homogenate; D, debris; E, endosomal fraction; L, lysosomal fraction; M, microsomes; C, cytosol.

Selective upregulation of cathepsin S activity in human keratinocytes and in HaCaT keratinocytes by IFN-γ Although keratinocytes do not express MHC class II molecules constitutively, exposure to IFN-γ can induce their expression thus enabling them to function as nonprofessional antigen-presenting cells. We therefore investigated the influence of this pro-inflammatory cytokine on MHC class I and II expression. Furthermore, we examined cathepsin activities of human keratinocytes in primary culture in comparison with those in the permanent human keratinocyte line HaCaT under these conditions.

The constitutive expression of MHC class I was increased by incubation with 1000 units per ml over a period of 72 h. Additionally, low levels of HLA-DQ and HLA-DP neo-expression along with a profound surface neo-expression of HLA-DR were detectable following IFN-γ stimulation. Moreover, strong intracellular staining for the invariant chain was also seen (**Fig 1**).

Interestingly, IFN-γ treatment (**Table III**) had similar effects on the activities of cathepsin B and NAG in both keratinocytes and HaCaT cells with slightly increased enzyme activities in whole cell homogenate and slightly decreased enzyme activities in endosomal and lysosomal fractions as compared with the controls. This tendency could be observed also in the case of cathepsin L. On the other hand, cathepsin S activity showed a very different behavior, which was similar for both cell types. Interestingly, cathepsin S activity showed a several-fold higher increase in whole cell homogenates (5-fold and 13-fold) than in endosomal and lysosomal fractions (2–3-fold). This different behavior of cathepsin S in comparison with that of the other lysosomal enzymes indicates the special function of cathepsin S in parallel to the upregulation of MHC class II molecules and invariant chain. This experiment further demonstrates that the permanent human keratinocyte line HaCaT may serve as a valuable tool to investigate immunologic characteristics of human keratinocytes.

In order to examine the mechanism of increased cathepsin S activity, we determined cathepsin S mRNA by reverse transcription–PCR in control keratinocytes in comparison with keratinocytes stimulated with IFN-γ (1000 U per ml) for 24 h. **Figure 2** shows that this cytokine induced mRNA formation, whereas in control cells cathepsin S mRNA was not detectable. Therefore, induction of cathepsin S after 24 h of treatment with IFN-γ was shown, which was measurable as a several-fold increase in enzyme activity after 72 h. In addition, we confirmed this induction at the protein level, not only in primary keratinocytes, but also in HaCaT keratinocytes (**Fig 3**). Whereas cathepsin S was found by immunoblotting in a crude organelle fraction of control HaCaT

Table II. Lysosomal enzyme activities in subcellular fractions of human keratinocytes (72 h control) in primary culture (KC) and syngeneic EBV-transformed B cells (B) derived from one patient[a]

	Cathepsin B		Cathepsin L		Cathepsin S		NAG	
	K	B	KC	B	KC	B	KC	B
H	21.8 ± 2.5	1.9 ± 0.3	0.2 ± 0.0	1.8 ± 0.1	0.01 ± 0.00	0.03 ± 0.00	44.6 ± 10.4	12.9 ± 0.5
D	24.9 ± 0.9	2.3 ± 0.1	0.6 ± 0.1	1.4 ± 0.0	0.01 ± 0.00	0.01 ± 0.00	89.2 ± 23.0	12.1 ± 0.1
E	73.0 ± 5.5	4.4 ± 0.4	13.3 ± 3.3	3.2 ± 0.7	0.06 ± 0.03	0.39 ± 0.10	130.9 ± 83.9	23.5 ± 2.1
L	269.0 ± 1.0	34.7 ± 2.1	11.6 ± 1.7	23.0 ± 5.0	0.15 ± 0.03	0.78 ± 0.21	247.7 ± 128.9	147.3 ± 26.6
M	7.3 ± 0.3	2.1 ± 0.9	0.3 ± 0.2	0.9 ± 0.3	ND	0.04 ± 0.00	24.4 ± 9.7	5.6 ± 0.5
C	5.6 ± 0.3	2.0 ± 0.5	0.2 ± 0.2	0.6 ± 0.0	ND	0.05 ± 0.01	20.3 ± 0.8	2.0 ± 0.1

[a]Units per g protein, mean of two values. In case of the B cells two experiments were used for calculation. For keratinocytes one experiment with two determinations was used. H, homogenate; D, debris; E, endosomal fraction; L, lysosomal fraction; M, microsomes; C, cytosol; ND, not detectable.

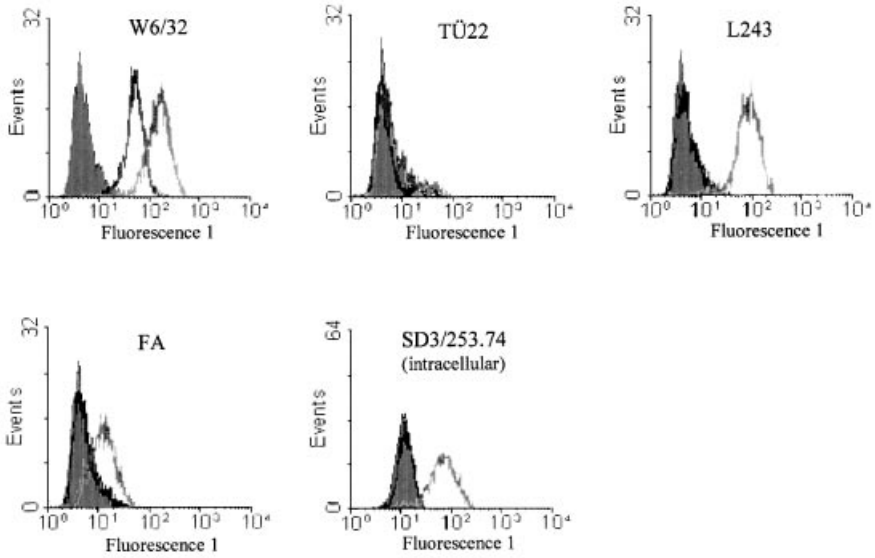

Figure 1. Neo-expression of MHC class II and invariant chain upon IFN-γ stimulaiton in human keratinocytes. Effect of IFN-γ stimulation (1000 U per ml, 72 h) on expression of MHC class I and II molecules and invariant chain of freshly isolated human keratinocytes. Filled profile = negative control; dark line = without IFN-γ stimulation; gray line = stimulated with IFN-γ.

Table III. Effect of IFN-γ stimulation (1000 U per ml, 72 h) on lysosomal enzyme activities in subcellular fractions of human keratinocytes in primary culture and HaCaT keratinocytes[a]

	Cathepsin B		Cathepsin L		Cathepsin S		NAG	
	KC	HaCaT	KC	HaCaT	KC	HaCaT	KC	HaCaT
H	154.7 ± 18.1	133.7 ± 4.1	231.5 ± 31.5	92.7 ± 47.4	1364.3 ± 1235.7	480.0 ± 41.7	116.9 ± 1.0	103.3 ± 21.5
E	96.2 ± 18.5	67.3 ± 15.8	78.6 ± 52.9	29.7 ± 30.6	282.5 ± 71.1	217.4 ± 48.0	75.6 ± 5.3	63.2 ± 20.3
L	111.5 ± 6.9	65.5 ± 3.0	156.5 ± 37.7	25.6 ± 35.6	255.1 ± 142.8	210.8 ± 48.6	86.2 ± 8.8	69.3 ± 25.8

[a]% of controls, mean of the values obtained from two experiments with at least two determinations each. In the first experiment keratinocytes from one patient were used (see **Table II**). The keratinocytes in the second experiment were collected from four patients. In this experiment lysosomal enzyme activities of the 72 h controls were in the same range as those of the first experiment. H, homogenate; E, endosomal fraction; L, lysosomal fraction.

cells, the amount of enzyme protein was too low to be detected in endosomal and lysosomal fractions of control primary keratinocytes; however, IFN-γ treatment for 72 h resulted in a clearly increased amount of cathepsin S protein in both HaCaT and primary keratinocytes. As expected lysosomal keratinocyte fractions did not contain the proform of cathepsin S (37 kDa), whereas in the endosomes the newly synthesized enzyme was present in a considerable amount as the enzymatically inactive proform.

DISCUSSION

Cathepsins are a family of lysosomal proteases involved in intracellular protein degradation. Consequently, most of the cathepsins are ubiquitously distributed and only some members show a restriction to certain tissues. Cathepsin S belongs to this group of restricted cathepsins, which is predominantly expressed in lymphatic tissues (Kirschke *et al*, 1986). We have found cathepsin S activity also in the spontaneously transformed human keratinocyte cell line HaCaT as well as in freshly isolated human keratinocytes. Endosomal/lysosomal cell compartments show, as expected, highest activities.

Furthermore, in general protein turnover cathepsins are also involved in the generation of antigenic peptides (Fineschi and Miller, 1997; Nakagawa and Rudensky, 1999; Villadangos *et al*, 1999). It is suggested that this incomplete proteolysis takes place in pre-lysosomal compartments (Authier *et al*, 1996); however, conflicting data exist regarding their exact role. Whereas Matsunuga *et al* (1993) were able to block primary immune responses by inhibition of cathepsin B, others demonstrated that

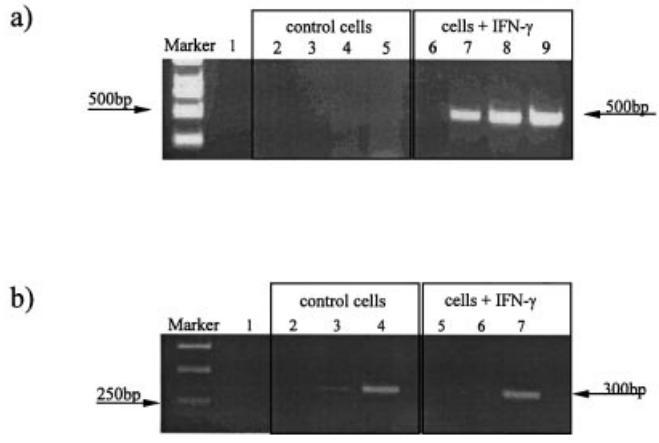

Figure 2. Induction of cathespin S in RNA in human keratinocytes upon IFN-γ stimulation. Reverse transcription–PCR of human cathepsin S (*a*) and β-actin (*b*). (*a*) 1 = negative control without RNA; 2 = negative control with 300 ng RNA/without reverse transcriptase; 3–5 = cDNA 100, 200, 500 ng; 6 = negative control with 300 ng RNA/without reverse transcriptase; 7–9 cDNA 100, 200, 500 ng. (*b*) 1 = negative control without RNA; 2 = negative control with 300 ng RNA and processed with DNase; 3 = negative control 300 ng RNA/without reverse transcriptase; 4 = 300 ng cDNA; 5 = negative control with 300 ng RNA with DNase processed; 6 = negative control 300 ng RNA without reverse transcriptase; 7 = 300 ng cDNA.

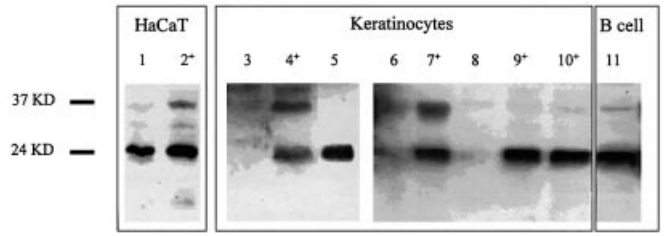

Figure 3. Induction of cathespin S protein in human keratinocytes upon IFN-γ stimulation. Western blot of human cathepsin S in HaCaT and primary keratinocytes in comparison with an EBV-transformed human B cell line (WT51). 1 = Organelles control, 2^+ = Organelles + IFN-γ, 3 + 6 = endosomal fraction control, $4^+ + 7^+$ = endosomal fraction + IFN-γ, 5 = cathepsin S, 8 = lysosomal fraction control, $9^+ + 10^+$ = lysosomal fraction + IFN-γ, 11 = lysosomal fraction of WT51.

antigen presentation is detectable also in mice deficient for either cathepsin B or S (Villadangos *et al*, 1997), thus arguing for the presence of alternative systems to take over the part of these cathepsins in antigen processing.

Keratinocytes are an integral part of the skin immune system. Although they do not express MHC class II molecules constitutively, exposure to IFN-γ can induce expression of these markers thus enabling them to function as nonprofessional antigen-presenting cells (Nickoloff and Turka, 1994). Our finding of the presence of high cathepsin B activity, lower cathepsin L activity, and very low cathepsin S activity indicates a more specific function of the latter. This may be an immunogenic function, which appears to be demonstrated by the relatively high cathepsin S activity in syngeneic B cells when compared with the activities of cathepsins B and L. Furthermore, the high ratio of endosomal to lysosomal cathepsin S activity in these professional antigen-presenting cells supports this immunogenic function. In endosomal compartments antigenic peptide loading is possible after the cleavage of invariant chain by the catalysis of cathepsin S (Riese *et al*, 1996). The concept is further supported by the selective increase in cathepsin S activity in HaCaT and primary keratinocytes, when MHC class II molecules are *de novo* expressed under the influence of IFN-γ. Under these conditions, the activities of the house-keeping proteinases, cathepsins B and L, were not increased. Interestingly, endosomal and lysosomal cathepsin S activities were changed very similarly in both primary and HaCat keratinocytes. The finding of 5–13-fold activity increases in whole cell lysates of both cell types points to a decrease of cytosolic proteinase inhibitors, which may be heterogeneous in the two preparations of primary keratinocytes examined. This may be substantiated by the finding of slightly increased activities of cathepsins B and L, but unchanged NAG activities. Whereas NAG is not known to be inhibited by cytosolic proteins, their inhibitory potential on cathepsins B and L is lower than that on cathepsin S (Abrahamson, 1994). These findings demonstrate that IFN-γ treatment of keratinocytes had differential effects on the activities on endosomal/lysosomal cathepsins B, L, and S as well as on their cytosolic inhibitors.

The mechanisms underlying the increase in cathepsin S activity upon stimulation of HaCaT and primary keratinocytes with IFN-γ for 72 h was found to be an increased expression of cathepsin S protein, which was found to be preceded by increased mRNA expression after 24 h of IFN-γ treatment. This induction parallels *de novo* expression of MHC class II molecules and invariant chain (Albanesi *et al*, 1998) and may be mediated through AP1 binding sites on the cathepsin S promoter (Shi *et al*, 1994). Whereas lysosomal cathepsin S activity appears to reflect the amount of cathepsin S protein, the endosomal fraction (composed of early and late compartments with different maturation states of cathepsins) contains both active cathepsin S and the inactive proform. Thus, in addition to the presence of the active enzyme a pool of preformed cathepsin S may be capable of being rapidly activated when cleavage of the invariant chain is required for maturation of MHC class II molecules within the endosomal compartment.

IFN-γ is believed to be a key cytokine in the induction phase of psoriasis (Szabo *et al*, 1998) where keratinocytes typically exhibit an MHC class II positive phenotype (Gottlieb *et al*, 1986). Several studies have demonstrated increased proteinase activities in psoriatic epidermis (Baird *et al*, 1990; Wiedow *et al*, 1992). More recently, processing of precursor forms of cathepsins L, B, and D to mature enzymes has been demonstrated in psoriasis (Kawada *et al*, 1997). As cathepsin S is involved in the proteolysis of the invariant chain (Riese *et al*, 1996) its induction might be of major importance in the context of antigen presentation. IFN-γ induced upregulation of cathepsin S activity in keratinocytes might therefore be an important feature in the pathogenesis of psoriasis where these cells are candidates to present a putative (auto-)antigen to T cells (Boehncke, 1996; Prinz *et al*, 1999).

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