

Bipartite regulation of different components of the MHC class I antigen-processing machinery during dendritic cell maturation

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Abstract

Dendritic cells (DC) are professional antigen-presenting cells (APC) which proceed from immature to a mature stage during their final differentiation. Immature DC are highly effective in terms of antigen uptake and processing, whereas mature DC become potent immunostimulatory cells. Until now, the expression profiles of the major components of the MHC class I antigen-processing machinery (APM) during DC development have not been well characterized. In this study, the mRNA and protein expression levels of the IFN- γ inducible proteasome subunits, of the proteasome activators PA28, and of key components required for peptide transport and MHC class I-peptide complex assembly have been evaluated in immature and mature stages of human monocyte-derived DC using semiquantitative RT-PCR and Western blot analyses. The IFN- γ -responsive immunoproteasome subunits LMP2, LMP7 and MECL1 are up-regulated in immature DC, whereas the other components of the MHC class I presentation machinery, such as PA28, TAP, tapasin, and HLA heavy and light chains, were found to be more abundant in mature DC. These findings support the hypothesis that immature DC produced by the differentiation of monocytes in response to IL-4 and granulocyte macrophage colony stimulating factor first increase their capacity to capture antigens and process them into peptides, thereby switching from housekeeping to immunoproteasomes, while mature DC rather up-regulate the components required for peptide translocation and MHC class I-peptide complex formation, and thus specialize in antigen presentation. Our results establish that MHC class I, like MHC class II surface expression, is markedly regulated during DC development and maturation.

Introduction

Dendritic cells (DC) are thought to be the most potent antigen-presenting cells (APC). Moreover, they play a crucial role in the stimulation of both primary and secondary CD4⁺ and CD8⁺ T cell responses (1,2). DC maturation can presently be obtained by culturing myeloid CD14⁺ or CD34⁺ precursors from peripheral blood or tissues in granulocyte macrophage colony stimulating factor (GM-CSF)-containing cytokine cocktails (3–7). *In vitro* DC culture systems have been used to study antigen capturing, processing and presenting in immature and mature DC. It has been shown that the immunostimulatory activity of DC is carefully regulated. Resting or immature DC are usually present in the periphery and are characterized by their potent ability for endocytosis, allowing antigen uptake and its processing to antigenic peptides (4,8),

but low capacity for binding and stimulating T cells. Several mechanisms have been reported to contribute to the proper function of immature DC, which include fluid-phase pinocytosis (9), phagocytosis (10), and the expression of endocytic receptors (11,12) and Fc γ receptors (Fc γ RII) (13). During their maturation process, immature DC migrate to lymphoid organs to present antigenic peptides via MHC molecules to T lymphocytes (2). Mature DC are less effective in antigen capturing and processing, but are highly specialized in presenting immunogenic peptides derived from encountered antigens and in activating naive T cells (14–16).

The potent antigen-presenting and T cell-stimulatory abilities of mature DC reflect several developmentally regulated changes, such as the up-regulation of the co-stimulatory

molecules CD40, CD80 and CD86, the secretion of cytokines modulating T cell activation, and the expression of new chemokine-like receptors (2,15–17). In addition, mature DC demonstrate increased expression of MHC class II surface antigen (15) resulting from at least two alterations. First, mature DC redistribute their MHC class II products from intracellular compartments to the plasma membrane. The MHC class II trafficking is controlled by the developmentally regulated peptide loading and the capacity of late endosomes and lysosomes to produce MHC class II–peptide complexes helping to coordinate antigen acquisition and inflammatory stimuli with the formation of TCR stimulatory ligands (18). Second, the maturation process is accompanied by the down-regulation of endocytosis leading to prolonged MHC class II surface expression and half-life, thereby resulting in an increased density of MHC class II–peptide complexes. However, comparable studies to define the critical role of the MHC class I antigen-processing machinery (APM) during DC maturation have not been carried out.

Although DC not only present exogenous antigens in the context of MHC class II molecules, but are also capable to cross-present exogenous antigens via the MHC class I antigen-processing pathway (19,20), studies defining the critical role of the MHC class I APM during DC maturation are still lacking. To further delineate the mechanisms underlying the conversion of immature to mature DC in terms of MHC class I-restricted antigen presentation, the mRNA and/or protein expression levels of the proteasome subunits LMP2, LMP7 and MECL1, of their activators PA28 α and PA28 β , of the peptide transporter subunits TAP1 and TAP2, of the chaperone tapasin, and of the HLA class I heavy chain (HC) and light chain [β_2 -microglobulin (β_2m)] molecules have been investigated during DC differentiation. The results indicate that the IFN- γ -inducible proteasome subunits appear to be involved in the antigen processing of immature DC, whereas PA28, TAP, tapasin, HLA class I HC molecules and β_2m are likely required for the antigen presentation in mature DC.

Methods

Leukapheresis and generation of DC

DC were generated from leukapheresis as previously described (5). In order to obtain mononuclear cells, the leukapheresis product was diluted and subjected to gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway). Following centrifugation, the interphase was harvested, washed 3 times in PBS without calcium or magnesium containing 1 mM EDTA (Biowhittaker, Walkersville, MD), and finally washed in complete RPMI 1640 medium (Biowhittaker) containing 20 μ g/ml gentamycin (Refobacin 10; Merck, Darmstadt, Germany), 2 mM glutamine (Biowhittaker) and 1% heat-inactivated (56°C for 30 min) autologous human plasma. Mononuclear cells were first plated at 5×10^7 /100-mm Petri dish in complete RPMI 1640 medium. After 1 h of culture, the non-adherent cells were discarded. On day 1, the culture medium was removed, and 10 ml of fresh complete medium containing 800 U/ml recombinant human GM-CSF (Leukomax; Novartis, Nürnberg, Germany) and 1000 U/ml recombinant human IL-4, kindly provided by Dr E. Liehl (Novartis Research

Institute, Vienna, Austria) was added. On day 3, a total of 1 ml medium was removed, and 3 ml complete medium supplemented with 8000 U GM-CSF and 10,000 U IL-4 were added. On day 5, the non-adherent cells were harvested, washed once in PBS, and then transferred into six-well culture plates at 5×10^5 cells/well in complete medium containing 800 U/ml recombinant human GM-CSF and 1000 U/ml recombinant human IL-4. After 24 h, a maturation cocktail mimicking monocyte-conditioned medium consisting of tumor necrosis factor- α (10 ng/ml; kindly provided by Dr Günther Adolf, Boehringer Ingelheim Austria, Vienna, Austria), prostaglandin E₂ (1 μ g/ml, Minprostin; Pharmacia & Upjohn, Erlangen, Germany), IL-1 β (10 ng/ml; Cell Concepts, Umkirch, Germany) and IL-6 (100 U/ml; a generous gift from Novartis, Basel, Switzerland) was added directly to all DC cultures (21,22).

Flow cytometry

For flow cytometric analysis, DC were stained with mAb directed against CD14 (Becton Dickinson, Brussels, Belgium) and CD83 (Immunotech, Marseilles, France). Isotype controls were run in parallel. Cell populations were analyzed on a FACScan (Becton Dickinson, Franklin Lakes, NJ) as previously described (5,23). Dead cells were gated out on the basis of their light scatter properties.

Primary allogeneic mixed leukocyte reaction (MLR)

The MLR was performed as described by Thurner *et al.* (5). Briefly, immature or mature DC at different concentrations were added in triplicates to 2×10^5 allogeneic T cells/well in 96-well flat-bottom plates and co-cultivated for 4–5 days in RPMI 1640 supplemented with gentamycin, glutamine and 5% allogeneic heat-inactivated human serum. For the last 12–16 h of co-incubation, [³H]thymidine (4 μ Ci final concentration/ml) was added and proliferation was determined by thymidine incorporation.

Semiquantitative RT-PCR analysis

The oligonucleotides used for PCR and Southern blot analysis were designed employing the Primer Designer program (version 2.01; Scientific & Educational Software, Durham, NC) and synthesized by the triester method (MWG Biotech, Ebersberg, Germany). The primers and the PCR conditions used are listed in Table 1. mRNA was extracted from 2.5×10^6 to 1×10^7 immature and mature DC obtained from different healthy volunteers using the QuickPrepMicro mRNA purification kit (Amersham Pharmacia Biotech, Freiburg, Germany). The mRNA concentration/ 2.5×10^6 cells varied during the DC development demonstrating ~2-fold increase in mature DC when compared to that of immature DC. mRNA was treated with RNase-free DNase I (Roche Diagnostics, Mannheim, Germany) for 1–4 h at 37°C in 25 mM Tris–HCl, 5 mM MgCl₂ and 0.1 mM EDTA (pH 7.2). mRNA was reverse transcribed using the by first-strand cDNA synthesis kit (Amersham Pharmacia Biotech). Cycle-per-cycle monitoring of PCR amplification was performed in a GeneAmp PCR System 2400 thermal cycler (Perkin-Elmer, Weiterstadt, Germany) to monitor the PCR amplification efficiency and to determine the exponential phase. Each set of PCR amplification contained β -actin as internal control. Final PCR conditions were in 50 ml 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 0.2 mM

Table 1. Primers used for PCR and hybridization

Genes	Primers (5' → 3')	Annealing temperature (°C)	Size (bp)	Oligonucleotides probes for hybridization	GenBank accession no.
Set 1					
TAP 1	f ccttcgttggtcagttatgcagcgg b tgcactggctatggtgagaatggac	61	302	gccgataccttcactcgaaacttaac	L21204
TAP 2	f cgtctcctttgcatatcccaatcgc b agccgccatcaccttatcatcttcg	61	322	tcaaggggctgacgtttac	AF105151
Tapasin	f tgcttgggatgatgatgagcc b tgacagagccatcggaatggtg	61	365	atcagggacaccttggggggtttg	AB010639
MECL1	f acttcatcgccccaaaatc b catagcctgcacagtttctcc	61	590	cgtccaagatggagctacac	Y13640
β-actin (1)	f tgtttgagaccttcaacacccacgc b cagcttctccttaatgtcacgcacg	61	281	aggatggcatgggggagggcatacc	X00351
Set 2					
LMP2	f atcatggcagtgagtttgacggg b ccaatggcaaaaggctgtcgagtc	60.2	374	agaggacttgtctgcacatctcatggtagc	U01025
LMP7	f cgtcttcccagagttgtgacagccaatc b ttccatcacctcaatctccagaggagtg	60.2	462	gccagacatggaggaggaggaagccaaga	D38048
β-actin (2)	f gcgtgacattaaggagaagctgtgc b ttgctgatccacatctgctggaag	60.2	450	aagacctgtacgccaacacagtgc	X00351
Set 3					
β ₂ -m	f aggctatccagcgtactccaaag b cggcaggcatactcatctttttc	58	247	gcattcagacttgtcttttcagc	AF072097
PA28α	f ggagccagctctcaatgaag b gcataccacgctcagagaa	58	417	gtggcccagtgaaactgcaatgaaaagatcg	AF078829
PA28β	f ggaggtcttccaggcagaatc b ataggctgcctcatctcgct	58	546	tggaaacagataagcaggagaagaa	NM002818
HLA-A2	f tatctgctggagccactccac b agactcaccgagtggaactg	58	289	ccagtacgcctacgacggcaaggattacat	X94570
β-actin (3)	f tcctgtggcatccacgaaact b gaagcatttgcggtggacgat	58	315	aagacctgtacgccaacacagtgc	X00351

Key: f = forward; b = backward.

dNTP, 0.5 μM each of the specific primers and 1.25 U Taq DNA polymerase (Perkin-Elmer). PCR cycling conditions for all amplifications were 94°C for 1 min, 61°C for 1 min and 72°C for 1 min. PCR products after 15, 20, 25 and 30 cycles of amplification were separated on a 1.5% agarose gel containing 0.4 μg/ml ethidium bromide and blotted on a nitrocellulose membrane (Roche Diagnostics). Filters were hybridized with biotin-labeled, specific oligonucleotide probes as listed in Table 1, and processed with a chemiluminescence-based Southern blot detection system (Roche Diagnostics) to achieve a high degree of sensitivity and specificity. Optical densities of target gene:β-actin signals were then recorded from scanned films and analyzed using a software program (Scion Image; Scion Corporation, Frederick, MD). Ratios of target gene:β-actin signals from the same cycle were calculated for comparative analysis and represent the relative transcriptional levels at the respective cycle step.

Western blot analysis

The following antibodies specific for various APM components were used: murine anti-TAP1 mAb 148.3 (24), murine anti-TAP2 mAb 2.17 and rabbit anti-tapasin polyclonal antibody (kindly provided by Dr F. Momburg, DKFZ, Heidelberg), murine anti-HLA-A, -B and -C mAb W6/32 (25), and the rabbit anti-LMP2 and anti-PA28β polyclonal antibodies (purchased from Affinity, Mamhead, UK).

For Western blot analysis, 5×10⁶ immature and mature DC

were lysed in 200 ml 1 SDS sample buffer containing 5% sucrose, 5% SDS and 60 mM Tris-HCl (pH 6.8), and cell debris was removed by centrifugation. The protein content of the cell lysates was determined by the Bradford method demonstrating ~2-fold increase in mature DC compared to immature DC (26). After incubation for 5 min at 95°C, 15 μg of total protein per lane was separated on a 8 or 16% SDS-PAGE gel respectively and transferred onto a nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany). Equal protein loading was verified by both staining the gel and the membrane with Coomassie brilliant blue and Ponceau S respectively. The membrane was incubated with specific antibody, followed by the appropriate horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse Ig (Dako, Hamburg, Germany). The signal of target protein was detected by the ECL detection system (Amersham Life Science, Braunschweig, Germany) and recorded on film in the linear detectable range. Area quantification was performed on the relevant band with a software program (Scion Image).

Results

Maturation of human CD14⁺ monocyte-derived DC

To analyze the expression levels of MHC class I APM components during DC maturation, monocyte-derived DC from peripheral blood mononuclear cells were generated using GM-

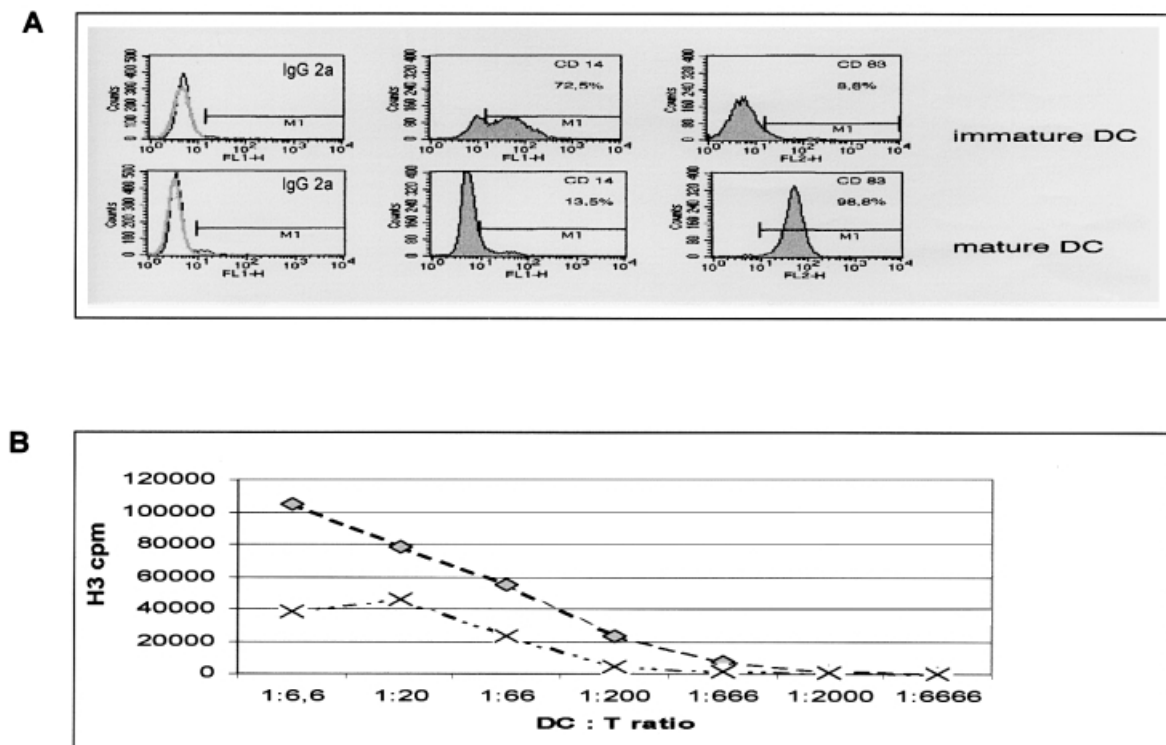


Fig. 1. The characterization of DC maturation. (A) Immature and mature DC were prepared as described in Methods. Flow cytometric analysis was performed using anti-CD14-FITC and anti-CD83-phyceorythrin mAb. The results are expressed as histograms. An isotype-matched antibody (IgG2a) was used as a control. (B) Allogeneic T cell proliferation was measured in DC/T cell co-cultivation experiments. Mature DC (diamonds), but not immature DC (crosses), exhibit strong allostimulatory potency in the primary allogeneic MLR.

CSF/IL-4-supplemented medium (5). Immature and mature DC displayed the characteristic DC phenotype in terms of morphology and of cell surface marker expression. Immature DC were CD14⁺ and CD83⁻, whereas mature DC were CD83⁺, but lacked CD14 expression (Fig. 1A). In addition, functional analyses revealed that mature DC exhibited a strong allostimulatory potential in primary allogeneic MLR, whereas immature DC lacked this property (Fig. 1B). Furthermore, mature DC also showed a reduced endocytic as well as antigen processing ability when compared to immature counterparts (data not shown; 11,23).

Down-regulation of immunoproteasome subunits LMP2, LMP7 and MECL1, and up-regulation of PA28 α and PA28 β during DC maturation

In order to define the underlying molecular mechanisms of increased antigen-processing ability in immature DC, the expression of the immunoproteasome subunits and their activators was determined both at the transcript and protein level. Semiquantitative RT-PCR and Western blot analyses were performed on equal concentrations of mRNA and protein preparations of CD14⁺-derived immature and mature DC. The mRNA expression pattern of the various APM components in immature and mature DC were normalized to β -actin mRNA expression, whereas their protein amounts were normalized on total protein content (Figs 2 and 3). The IFN- γ treated B-lymphoblastoid cell line T1 expressing high levels of MHC class I APM molecules served as a positive control for all

semiquantitative RT-PCR and Western blot analyses (27). As shown in Fig. 2, the transcription of the immunoproteasome subunits LMP2, LMP7 and MECL1 in mature DC was down-regulated 1.6-, 3.4- and 5.0-fold respectively when compared to that of immature DC. In contrast, a 3.0- and 5.3-fold up-regulation of PA28 α and PA28 β mRNA was found in mature DC. This discrepancy of down-regulated immunoproteasome subunits and up-regulated proteasome activators was further confirmed by Western blot analysis in a linear detectable range as representatively shown for LMP2 and PA28 β proteins, which were down-regulated 2.4-fold and up-regulated 2.5-fold, respectively (Fig. 4). Thus, a differential expression profile of the immunoproteasome subunits and the proteasome activators was detected during DC maturation.

Up-regulation of peptide transporter TAP, tapasin and MHC class I molecules during DC maturation

To further elucidate the molecular mechanisms of potent antigen-presenting and T cell-stimulatory ability in mature DC, the differential regulation of peptide transporter subunits TAP1, TAP2, tapasin, HLA class I molecules and β_2 m was subsequently monitored by semiquantitative RT-PCR and Western blot analysis. mRNA expression for TAP1, TAP2, tapasin, HLA class I HC and β_2 m in mature DC was up-regulated 4.2-, 2.0-, 3.5-, 4.2- and 2.8-fold respectively in comparison to that in immature DC (Fig. 3). This enhanced transcription was accompanied by an up-regulation of protein expression as demonstrated by Western blot analysis. In

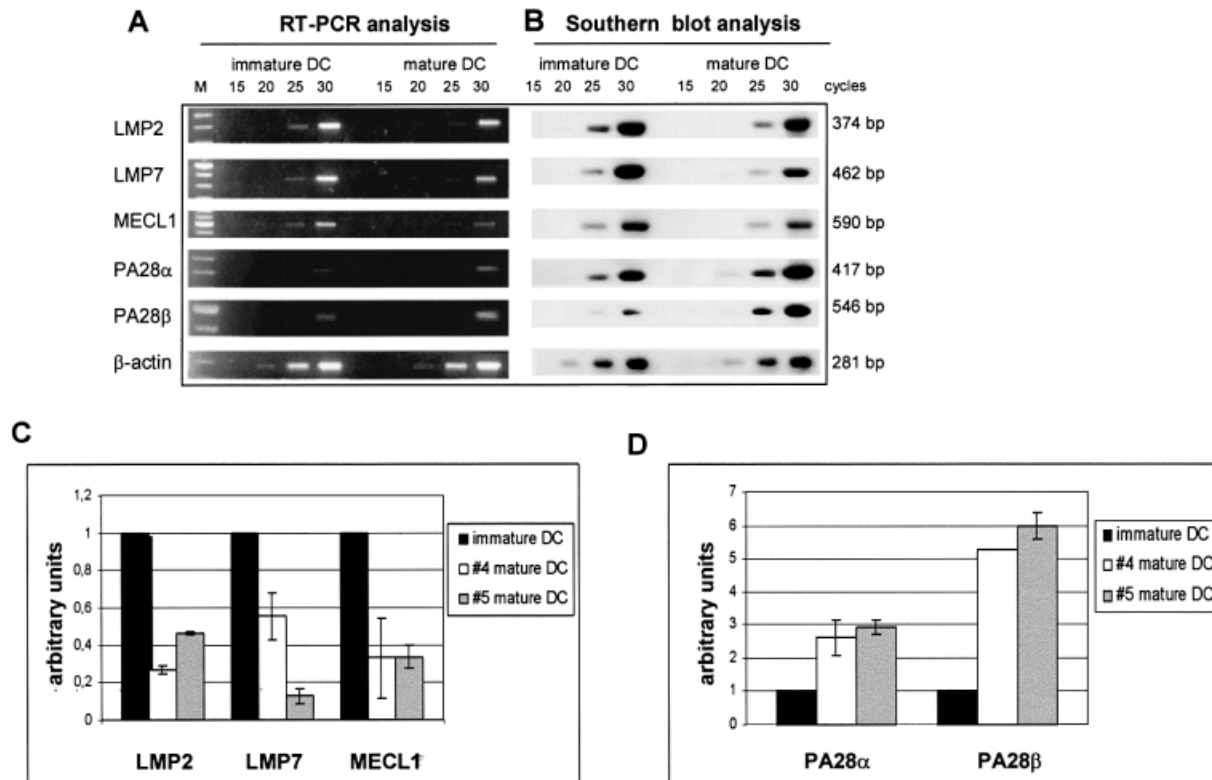


Fig. 2. Semiquantitative RT-PCR analysis of the mRNA expression of immunoproteasome subunits and their activators in immature and mature DC. (A) Cycle-per-cycle monitoring of RT-PCR was performed on mRNA from CD14⁺ monocyte-derived DC from two healthy volunteers (#4 and #5). β -Actin was used as an internal control for RT-PCR. (B) PCR products were blotted and hybridized with specific probes. Target and β -actin gene signals were detected using a chemiluminescence-based Southern blot detection system and recorded on film for optical density measurement. (C and D) Down-regulation of LMP2, LMP7 and MECL1 mRNA expression, and up-regulation of PA28 α and PA28 β mRNA expression during DC maturation. The data are presented as follows: ratios of target gene optical density: β -actin optical density from the same cycle were calculated and represented the relative transcriptional level of target gene at the respective cycle step. For comparative analysis, ratios of target gene: β -actin in immature DC were expressed as 1 arbitrary unit. Results summarize the representative data of at least two independent experiments using DC derived from two healthy volunteers.

mature DC, a 5.4-, 3.4-, 4.7- and 2.9-fold increase of TAP1, TAP2, tapasin and HLA class I HC protein expression was found respectively (Fig. 4), suggesting that these APM components were strongly up-regulated during DC maturation.

Discussion

Many lines of evidence have postulated that DC can present exogenous antigens on both MHC class I and II molecules (4,19,28,29). Immature, but not mature DC are highly effective in processing native protein antigen for both MHC class I and II presentation (4,8). Furthermore, mature DC are more potent in antigen presentation to both CD4⁺ and CD8⁺ T cells (30–33).

Although the function of DC has been well characterized during the last decade, the molecular regulation of processing and presentation in the MHC class I pathway during DC development is still not well understood. Accumulating evidence supports the hypothesis that the immunoproteasome subunits LMP2, LMP7 and MECL1 are required for effective antigen processing of immunodominant T cell epitopes by improving the proteolytic production of antigenic peptides (34–36). Impaired MHC class I antigen presentation has been

detected in various human tumors and attributed to the down-regulation or lack of immunoproteasome subunit expression (37,38). The present study shows for the first time regulation of various APM components during DC development and a bipartite regulation of the different molecules. During the differentiation process, DC first develop the machinery required for the effective processing of antigenic compounds and then the proteins needed for transport (TAP) and assembly (MHC and tapasin) of the MHC class I-peptide complex. An up-regulation of the proteasome subunits was found in immature DC when compared to mature DC, although the distinct expression levels of the LMP subunits represent the smallest change of all antigen-processing components analyzed. The observed switch in the proteasome subunit expression might contribute to the enhanced antigen-processing capability of immature DC. The present finding is not consistent with a recent report demonstrating an increased incorporation of LMP2, LMP7 and MECL1 in immunoprecipitated proteasomes of mature DC (39). This, however, does not necessarily reflect higher protein expression levels of these subunits. Therefore, the discrepancy on the immunoproteasome regulation during DC maturation might be explained by the different methods used. In contrast to the immuno-

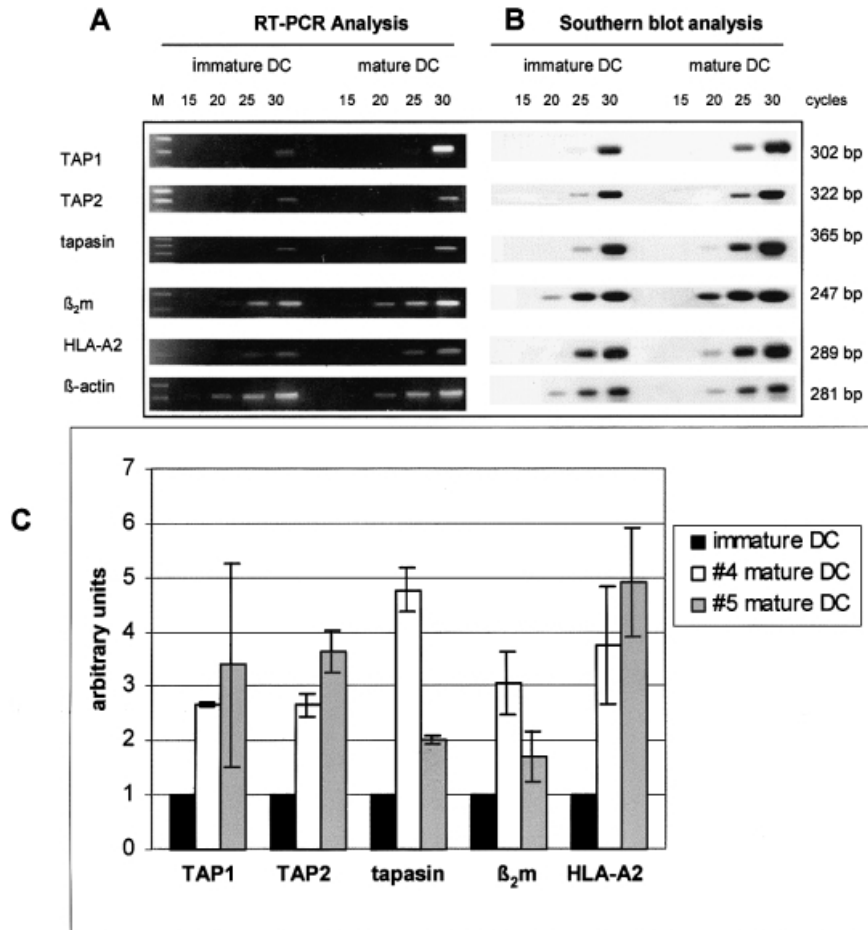


Fig. 3. Semiquantitative RT-PCR analysis of the peptide transporter, tapasin, HLA-class I and β_2m in immature and mature DC. (A) Cycle-per-cycle monitoring of RT-PCR were performed on mRNA from CD14⁺ monocyte-derived DC of two healthy volunteers. β -Actin was used as an internal control for RT-PCR. (B) PCR products were blotted and hybridized with specific probes. Target and β -actin gene signals were detected using a chemiluminescence-based Southern blot detection system and recorded on film for optical density measurement. (C) The gene expression of TAP1, TAP2, tapasin, β_2m and HLA- α HC was up-regulated. Results of at least two independent experiments are represented as described in Fig. 2.

proteasome subunits, the mRNA and protein expression of the proteasome activator PA28 α and PA28 β were up-regulated during DC maturation, which is in line with Macagno *et al.* (39). Since the PA28 subunits are required for the incorporation of the immunosubunits into the proteasome (30), the enhancement in the immunoproteasome/constitutive proteasome ratio might be mediated by a PA28-dependent immunoproteasome assembly. In contrast, the bipartite regulation of the proteasome activators and the immunoproteasome subunits during DC maturation described in this manuscript argues for a distinct role of these components. PA28 can enhance antigen presentation by altering the cleavage characteristics of the 20S proteasome (34). In addition, different effects on the MHC class I APM pathway by PA28 and immunoproteasome subunits have been described (36,41). In line with these reports, the present findings suggest that PA28 might mediate an enhanced MHC class I presentation independent of immunoproteasomes, although the exact mechanism by which PA28 exerts its function in mature DC remains to be determined.

In contrast to the down-regulation of immunoproteasome subunits, both mRNA and protein expression levels of peptide transporter TAP1 and TAP2 were up-regulated during DC maturation. Although little is presently known about the regulation of TAP1 and TAP2 in DC, many studies have demonstrated key roles for TAP in regulating MHC class I antigen presentation (38,42). Furthermore, recent experiments also showed that TAP is essential for the processing of the ovalbumin antigen in DC (43,44). The up-regulation of TAP1 and TAP2 found in this study supports the hypothesis that TAP is required for the potent antigen-presenting ability of mature DC by improving the peptide transport rate into the endoplasmic reticulum and subsequently the amount of peptides available for MHC class I-peptide assembly.

Studies on deficient cell lines and knock out mice revealed that the chaperone tapasin plays an important role in the formation of MHC class I-peptide complex by retaining class I heterodimers in the endoplasmic reticulum until optimal ligand selection is completed (45,46). So far, no reports on the regulation of tapasin during DC development have been

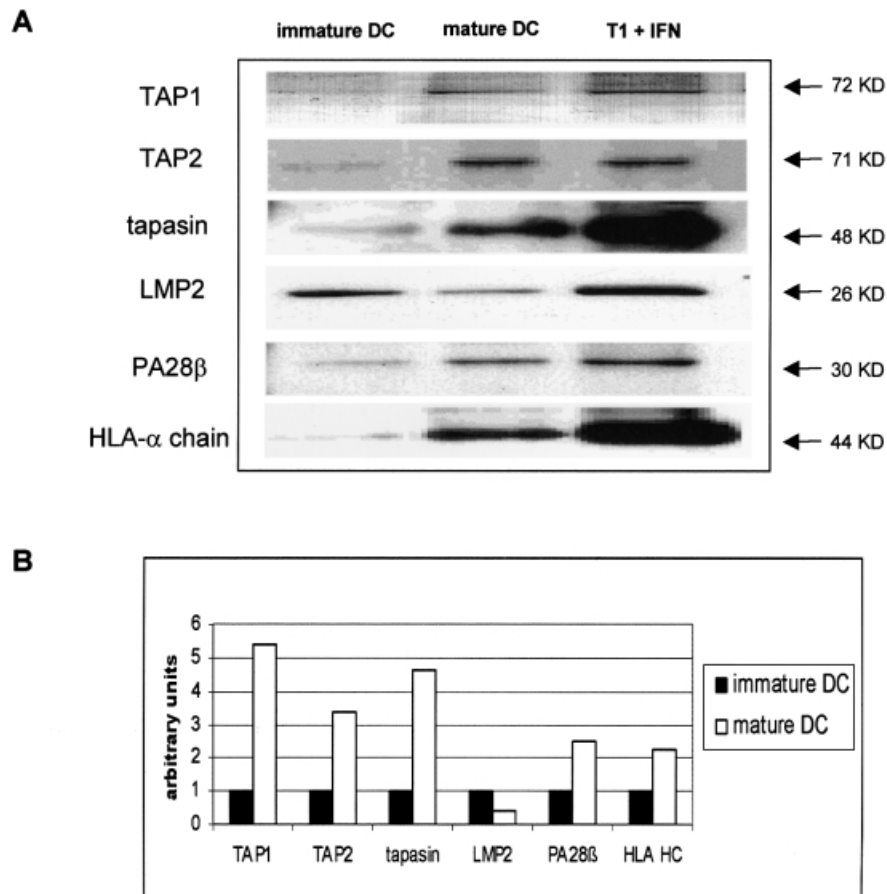


Fig. 4. Heterogeneous protein expression of various APM components during DC maturation. (A) Total protein (15 µg) from immature DC, mature DC and IFN-treated T1 cells was separated on a SDS-PAGE gel and transferred onto a nitrocellulose membrane. The membranes were probed with the anti-LMP2 and anti-PA28β polyclonal antibodies, anti-TAP1 mAb 148.3, anti-TAP2 mAb 2.17, rabbit anti-tapasin polyclonal antibody, and anti-HLA-A, -B and -C mAb W6/32 respectively, followed by a horseradish peroxidase-conjugated secondary antibody. The target proteins were revealed by chemiluminescence. (B) The protein expression of TAP1, TAP2, tapasin, PA28β and HLAα chain were up-regulated. LMP2 protein was down-regulated during DC maturation. Area quantification was performed on the relevant band in the linear detectable range and represented the relative expression level of target protein. For comparative analysis, optical density values of target proteins in immature DC were expressed as 1 arbitrary unit. Results are representatives of at least two independent experiments.

shown. The significant up-regulation of tapasin mRNA and protein expression shown in this study suggests that proper tapasin expression is associated with increased antigen presentation in mature DC.

In contrast to TAP and tapasin, the up-regulation of MHC class I molecules has been extensively analyzed in mature DC and appears to be a major marker of DC maturation. The present findings show an up-regulation of HLA class I HC at the mRNA and protein levels during DC maturation along with an increase of β₂m expression. The latter observation is in accordance with a recent report demonstrating an up-regulation for β₂m in human monocyte-derived mature DC (48). Promising results of animal studies and preliminary clinical trials have supported the successful implementation of DC-based immunotherapies (49–51). However, whether mature or immature DC should be employed for such approaches has not yet been defined. The data described here strongly suggest that the generation of an effective antitumor immune response depends on the strategy to deliver the antigenic epitope into immature or mature DC.

This is further strengthened by Jonuleit and coworkers (52), demonstrating that the maturational state of DC influences the priming and differentiation of T cells. In a recent study using immature DC, the ovalbumin antigen expressed as a bacterial cytoplasmic fusion protein was presented 10⁶ times more efficiently via the exogenous pathway on MHC class I molecule than soluble ovalbumin protein. This exogenous pathway in immature DC requires a transport from the phagolysosome to the cytosol before the antigenic protein is processed via the classical MHC class I APM pathway (43). To elucidate the molecular mechanisms of the potential transport from phagolysosome into cytosol in immature DC is of great interest for optimizing DC-based immunotherapeutic approaches.

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Abbreviations

APC	antigen-presenting cell
APM	antigen-processing machinery
β_2m	β_2 -microglobulin
DC	dendritic cell
GM-CSF	granulocyte macrophage colony stimulating factor
HC	heavy chain
LMP	low molecular weight proteins
MECL1	multicatalytic endopeptidase complex-like 1
MLR	mixed lymphocyte reaction
PA	proteasome activator
TAP	transporter associated with antigen processing

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