

# Inhibition of CD83 Cell Surface Expression during Dendritic Cell Maturation by Interference with Nuclear Export of CD83 mRNA

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## Abstract

Dendritic cells (DCs), nature's adjuvant, must mature to sensitize T cells. However, although the maturation process is essential, it is not yet fully understood at the molecular level. In this study, we investigated the course of expression of the unique hypusine-containing protein eukaryotic initiation factor 5A (eIF-5A), which is part of a particular RNA nuclear export pathway, during *in vitro* generation of human DCs. We show that eIF-5A expression is significantly upregulated during DC maturation. Furthermore, an inhibitor of the hypusine modification, GC7 (*N*<sup>1</sup>-guanyl-1,7-diaminoheptane), prevents CD83 surface expression by apparently interfering with nucleocytoplasmic translocation of the CD83 mRNA and, importantly, significantly inhibits DC-mediated T lymphocyte activation. The data presented suggest that CD83 mRNA is transported from the nucleus to the cytoplasm via a specific nuclear export pathway and that hypusine formation appears to be essential for the maturation of functional DCs. Therefore, pharmacological interference with hypusine formation may provide a new possibility to modulate DC function.

Key words: dendritic cells • CD83 • hypusine • eIF-5A • nuclear export

## Introduction

Dendritic cells (DCs)<sup>1</sup> are specialized to sensitize helper and killer T cells and thus act as "nature's adjuvant" in inducing T cell-mediated immunity (1). Immature DCs capture antigens in the periphery but lack full T cell-stimulatory capacity. In the presence of appropriate stimuli (such as microbial products and/or inflammatory cytokines), the DCs then mature. DCs upregulate T cell adhesion and costimulatory molecules as well as selected chemokine receptors that guide DC migration into secondary lymphoid organs for priming of antigen-specific T cells (2). DCs are defined by their po-

tent T cell-stimulatory capacity (e.g., in the allo-MLR) as well as a characteristic morphology (nonadherent cells with motile veils) and phenotype (upregulation of CD86 and *de novo* expression of CD83; references 3 and 4).

DCs can be generated *in vitro* either from rare proliferating CD34<sup>+</sup> or frequent, nonproliferating CD14<sup>+</sup> monocytic precursors (5–8). The generation of DCs from monocytes under the use of GM-CSF and IL-4 yields homogenous DC progenitors that are well suited for studying the maturation of DCs *in vitro* (9).

Eukaryotic initiation factor 5A (eIF-5A) is the only cellular protein known to contain the unusual amino acid hypusine, a modification that appears to be required for cell proliferation (10, 11). The hypusine modification is a spermidine-dependent posttranslational reaction that is catalyzed by two enzymes. This includes the transfer of the aminobutyl moiety of spermidine to the  $\epsilon$ -NH<sub>2</sub> group of lysine at position 50 in eIF-5A by deoxyhypusine synthase (12–14). The intermediate that is generated is subsequently hydroxylated by deoxyhypusine hydroxylase (15), resulting

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<sup>1</sup>Abbreviations used in this paper: AREs, AU-rich elements; DCs, dendritic cells; eIF-5A, eukaryotic initiation factor 5A; ERG, early response gene; hnRNPs, heterogeneous nuclear ribonucleoproteins.

in the active form of eIF-5A. Although eIF-5A was originally designated as an "initiation factor" (16, 17), more recent *in vitro* and *in vivo* data have demonstrated that eIF-5A is not an initiator of protein translation (18–20). In fact, the subsequent finding that eIF-5A is a cellular cofactor of HIV-1 Rev and HTLV-I Rex transactivator proteins suggested an entirely different eIF-5A activity (21–24). Both Rev and Rex are nucleocytoplasmic shuttle proteins that mediate the nuclear export of incompletely spliced and unspliced viral mRNAs (25, 26). Thus, eIF-5A appears to be part of a specific nuclear export pathway that is exploited by the Rev/Rex class of retroviral RNA transport factors. This notion is further supported by the finding that, in yeast, eIF-5A affects the decay of specific mRNAs that are transported from the nucleus to their cytoplasmic site of degradation (20, 27) and, more recently, that eIF-5A is a high-copy suppressor of transport-deficient TATA binding protein mutants (28). Furthermore, the eIF-5A protein itself has been shown to accumulate at the nucleoplasmic site of nuclear pore complexes to interact with the general nuclear export receptor CRM1 and to translocate from the nucleus to the cytoplasm in mammalian cells (29).

Investigation of eIF-5A mRNA levels in human cells revealed that eIF-5A is constitutively expressed in cell lines as well as in various tissues (30). In contrast, the eIF-5A gene appears to be subject to distinct regulation in primary lymphoid cells. In particular, eIF-5A gene expression is constitutively low but inducible with T lymphocyte-specific stimuli in human PBMCs (30). The combined data suggest that the hypusine-containing protein eIF-5A fulfills a specific and presumably essential function during activation and/or proliferation of primary human blood cells.

In this work, we investigated the expression level of the hypusine-containing protein eIF-5A during maturation of primary human DCs. Using an inhibitor of hypusine modification, we are able to show that formation of hypusine is required for the expression of the DC-specific molecule CD83 and the full stimulatory activity of mature DCs, demonstrating a potentially novel approach by which to interfere with DC function.

## Materials and Methods

**Cell Culture Medium.** Cells were cultured using a standard medium (referred to as 1% human plasma medium), which consisted of RPMI 1640 (BioWhittaker) supplemented with glutamine (300  $\mu$ g/ml) (BioWhittaker), penicillin/streptomycin (20  $\mu$ g/ml), 10 mM HEPES, pH 7.5 (Sigma-Aldrich), and 1% heat-inactivated (56°C; 30 min) human plasma from a single AB donor, obtained from the Department of Transfusion Medicine, Erlangen, Germany.

**Generation of DCs.** PBMCs ( $5 \times 10^7$ ) were isolated from buffy coats by sedimentation in Ficoll-hypaque (Amersham Pharmacia Biotech) and seeded onto IgG-coated (10  $\mu$ g/ml  $\gamma$ -globulin from Cohn fraction; Sigma-Aldrich) 100 mm-culture dishes and incubated at 37°C in 5% CO<sub>2</sub>. After 1 and 7 h of incubation, nonadherent cell fractions were harvested, and the remaining adherent cells were further cultured in 1% human plasma medium

supplemented with the cytokines GM-CSF (800 U/ml) and IL-4 (1,000 U/ml). Fresh medium (5 ml) containing 4,000 U GM-CSF and 5,000 U IL-4 was added to the culture dish at day 3 of this incubation period. On day 4 or 5, nonadherent cells were collected, counted, and transferred into new dishes at a density of  $0.3\text{--}0.5 \times 10^5$  cells/ml. For final DC maturation, 1% human plasma medium was supplemented with TNF- $\alpha$  (25 ng/ml), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>; 1 ng/ml), GM-CSF (400 U/ml), and IL-4 (500 U/ml) (31). In the studies using the hypusine inhibitor GC7 (N<sup>1</sup>-guanyl-1,7-diaminoheptane), immature DCs were pretreated at day 4 or 5 with GC7 at a concentration of 1  $\mu$ M for 10 min before addition of the final maturation medium.

**Cytokines and GC7.** Recombinant human (rh)GM-CSF and GC7 (32) were obtained from Novartis Research Institute, rhIL-4 from Genzyme, rhTNF- $\alpha$  from Boehringer, and PGE<sub>2</sub> from Cayman Chemical.

**FACS<sup>®</sup> Analyses.** For flow cytometry analyses, mAbs recognizing the following antigens were used: CD83 (Immunotech), CD80, CD86, CD13, CD68, MHC class I, MHC class II, and CD95 (Becton Dickinson). The isotype controls IgG1a and IgG2b were obtained from Becton Dickinson and were run in parallel. Cell populations were phenotyped with the panel of mAbs listed above and analyzed on a FACScan™ (Becton Dickinson) as described previously (8). Nonviable cells were gated out on the basis of their light scattering properties.

**Western Blot Analyses.** Cells ( $0.5 \times 10^6$ ) were harvested on days 4, 5, 6, and 7 of the DC generation procedure, washed with PBS, and solubilized in gel loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5%  $\beta$ -mercaptoethanol, and 0.1% bromophenol blue). After SDS-PAGE, the separated proteins were transferred onto nitrocellulose membranes, and specific antibodies were used to detect eIF-5A (33), histone H1 (StressGen Biotechnologies), and CD83 as described previously (29).

**RNA Analyses.** CD83 and eIF-5A mRNA levels were analyzed by PCR. Total cellular RNA from  $10^6$  cells was isolated at different time points of DC maturation using TRIzol™ Reagent (GIBCO BRL). Subsequently, the RNA was reverse transcribed into single-stranded cDNA using AMV reverse transcriptase according to the manufacturer's protocol (Roche Molecular Biochemicals). Serial dilutions of the cDNA preparations were subjected to AmpliTaq® (Perkin-Elmer) DNA polymerase-mediated PCR amplification using primer pairs specific for eIF-5A (5'-GCAGATGACTTGGACTTCGAGACAGG-3' and 5'-CCT-TGATTGCAACAGCTGCCTCCTC-3') and CD83 (5'-GTT-ATTGGAGGGTGGTGAAGAGAGG-3' and 5'-GTGAGGAGT-CACTAGCCCTAAATGC-3'). Amplification of the mRNA coding for the ribosomal protein S14 served as an internal control (5'-GGCAGACCGAGATGAATCCTCA-3' and 5'-CAGGTC-CAGGGTCTTGGTCC-3'). The profile for amplification involved 30 cycles of denaturation at 95°C for 60 s, primer annealing at 55°C for 60 s, and primer extension at 72°C for 90 s. The reaction products were analyzed on ethidium bromide-stained 2% agarose gels.

Northern blot analysis of 5  $\mu$ g of total cellular RNA derived from either DCs or HeLa cells was performed as described previously (34). CD83-specific transcripts were detected using the radiolabeled synthetic oligonucleotide probe 5'-TCTCCATCCTCTCTTACCACCCTCCAATAAC-3'. To control for comparable RNA amounts, the filters were stripped and rehybridized using a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) probe, 5'-CCATGGTGGTGAAGACGCCAGTGGACTCC-3'.

**Immunofluorescence Microscopy.** 2 d after addition of the final maturation cocktail, cells were harvested and washed with RPMI

medium. Subsequently,  $0.5 \times 10^6$  cells were spun onto polylysine-coated microslides (Menzel-Gläser) for 30 s at 300 rpm using a cytospin3 centrifuge (Shandon), fixed in 2% paraformaldehyde (Merck), and washed three times with PBS and 1% glycerol (Sigma-Aldrich). Blocking was performed with 1% BSA (Sigma-Aldrich). Cells were then incubated with primary anti-CD83 mAb, followed by secondary Cy3-conjugated, affinity-purified goat anti-mouse IgG (Rockland). Reactions were performed for 30 min at ambient temperature. Finally, the slides were washed five times with PBS, and cells were embedded in Moviol (Calbiochem). The samples were analyzed using a ZEISS Axiovert-135 microscope. Images were recorded with a cooled MicroMax CCD camera (Princeton Instruments) and processed using the IP-Lab spectrum and Adobe Photoshop software.

**In Situ Hybridization.** 12 h after addition of the final maturation cocktail,  $0.5 \times 10^6$  untreated or GC7-treated cells were seeded onto adhesion slides (Bio-Rad) and fixed for 15 min with 3% paraformaldehyde. After washing with PBS, cells were permeabilized with 0.5% Triton X-100 for 5 min, washed twice with PBS, and incubated twice for 10 min with  $2 \times$  SSC. For RNA detection, the following digoxigenin-labeled oligonucleotides from Eurogentec were used: CD83, 5'-TGGTTCCTTCGACGC-3' and 5'-TGTGGACTTGCCCTG-3'; CD86, 5'-ACTGACAAG-ACGCGG-3' and 5'-CAAGTATATGGGCCG-3'. For hybridization, cells were incubated in hybridization solution containing 10 ng/ $\mu$ l of each labeled oligo, 25% deionized formamide, 1  $\mu$ g/ $\mu$ l *Escherichia coli* tRNA (Sigma-Aldrich),  $2 \times$  SSC, 0.5% BSA, and 10% dextran sulfate for 16 h at 42°C in a humidified chamber. Unbound probe was removed by two 30-min washes in  $2 \times$  SSC and one 15-min wash in  $1 \times$  SSC. Subsequently, cells were washed with 0.1% Triton X-100 in PBS and blocked with 1% BSA for 30 min. Hybrids were stained for 30 min with primary  $\alpha$ -digoxigenin mAb (Roche Molecular Biochemicals). After extensive wash steps in PBS, cells were incubated with the appropriate secondary antibody coupled to Cy3 fluorophore (Biotrends). The cells were then treated with 1  $\mu$ g/ml DAPI (4',6-diamidino-2-phenylindole; Roche Molecular Biochemicals) in PBS, washed again several times in PBS, and mounted in Mowiol. Samples were analyzed by immunofluorescence microscopy as described above.

**Allogeneic MLR.** CD4<sup>+</sup> and CD8<sup>+</sup> T cells were isolated from buffy coats and stimulated with mature allogeneic DCs, which were either untreated or pretreated with GC7 (end concentration of 1  $\mu$ M) during DC maturation. T cells ( $2 \times 10^5$  per well) and DCs were cocultivated for 4 d in 200  $\mu$ l RPMI, supplemented with 5% human serum from a single AB donor in 96-well cell culture dishes. Then, cells were pulsed with [<sup>3</sup>H]thymidine (1  $\mu$ Ci/well; Amersham Pharmacia Biotech) for 8–16 h. The culture supernatants were harvested onto glass fiber filtermates using an IH-110 harvester (Inotech), and filters were counted in a 1450 microplate counter (Wallac).

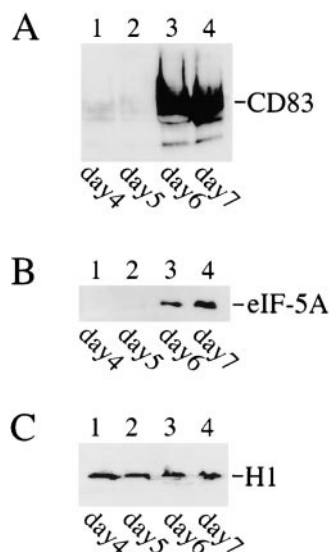
## Results

**The Hypusine Inhibitor GC7 Affects CD83 Surface Expression in DCs.** To investigate eIF-5A expression during DC maturation, we generated human DCs in vitro, following a modified two-step protocol (31). The mature DCs generated by this protocol displayed the specific parameters that are routinely used to define mature DCs. Mature DCs are characterized by the typical morphology of nonadherent cells, a distinct cytofluorographic profile (HLA-DR<sup>+++</sup>CD86<sup>++</sup>

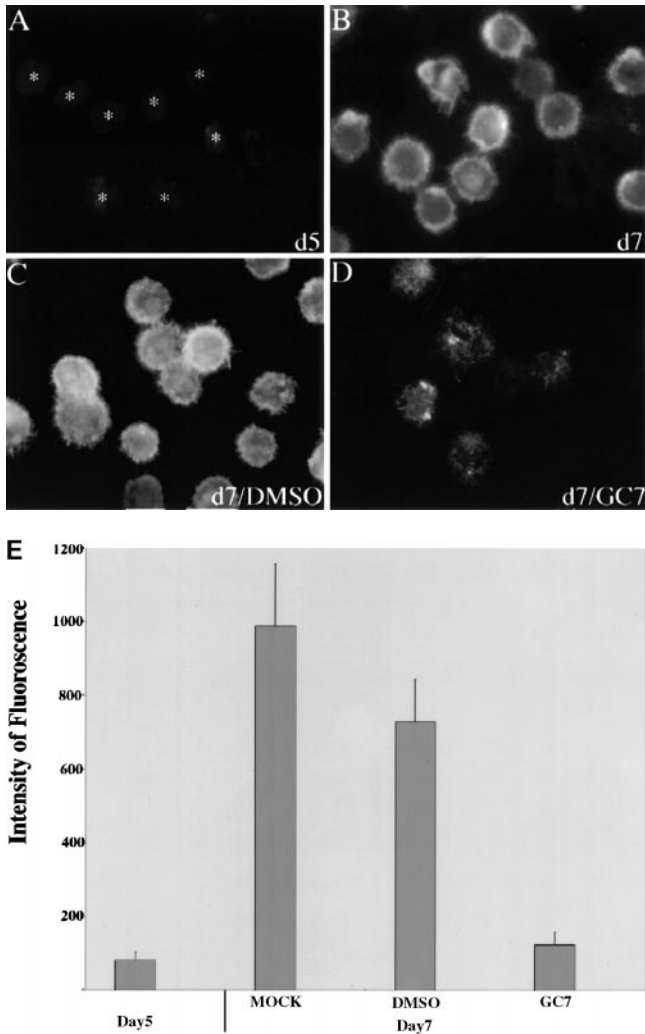
CD14<sup>-</sup>CD83<sup>+++</sup>p55<sup>++</sup>), and a highly potent stimulatory capacity in allogeneic MLRs at DC/T cell ratios of  $\leq 1:300$ . Furthermore, these cells stably maintained the DC phenotype when cultured for an additional 1–2 d in the absence of exogenously added cytokines (also known as the “wash-out” or “stability” test; reference 8).

We first examined eIF-5A levels by Western blot analysis using total protein extracts from various stages of DC generation. As shown in Fig. 1 B, the eIF-5A protein, migrating at a relative molecular mass of  $\sim 18$  kD (35, 36), was barely detectable within the initial days of cell culture (lanes 1 and 2). Clearly, a significant increase in eIF-5A protein was detected toward the end, namely at days 6 and 7, of the maturation period (Fig. 1 B, lane 3 and 4). As eIF-5A may be required for functional expression of DC-specific molecules, identical protein blots were subsequently probed with antibodies directed against CD83, a molecule of unknown function that is, nonetheless, one of the best cell surface markers for mature DCs (37). These analyses demonstrated that the time course of CD83 protein expression followed similar kinetics to those of eIF-5A during DC maturation (compare Fig. 1, A and B). This observation could mean that the expression of CD83 may depend on eIF-5A function.

The 154-amino acid eIF-5A protein is unique because it is the only cellular protein known to date to contain the unusual amino acid hypusine (10, 11). This enzyme-catalyzed posttranslational modification is essential for eIF-5A function and can be blocked in cell culture with deoxyhypusine synthase inhibitors (38–40). In particular, the low-molecular-mass drug GC7 has been shown to be the most specific and potent inhibitor, with a  $K_i$  value  $< 10$  nm (32, 38). Therefore, we next investigated the effect of GC7 on CD83 surface expression. As seen in Fig. 2, CD83-specific immunofluorescence analysis revealed that although CD83 surface expression was absent on day 5, it was easily detectable on day 7 of DC maturation (compare panels A and B),

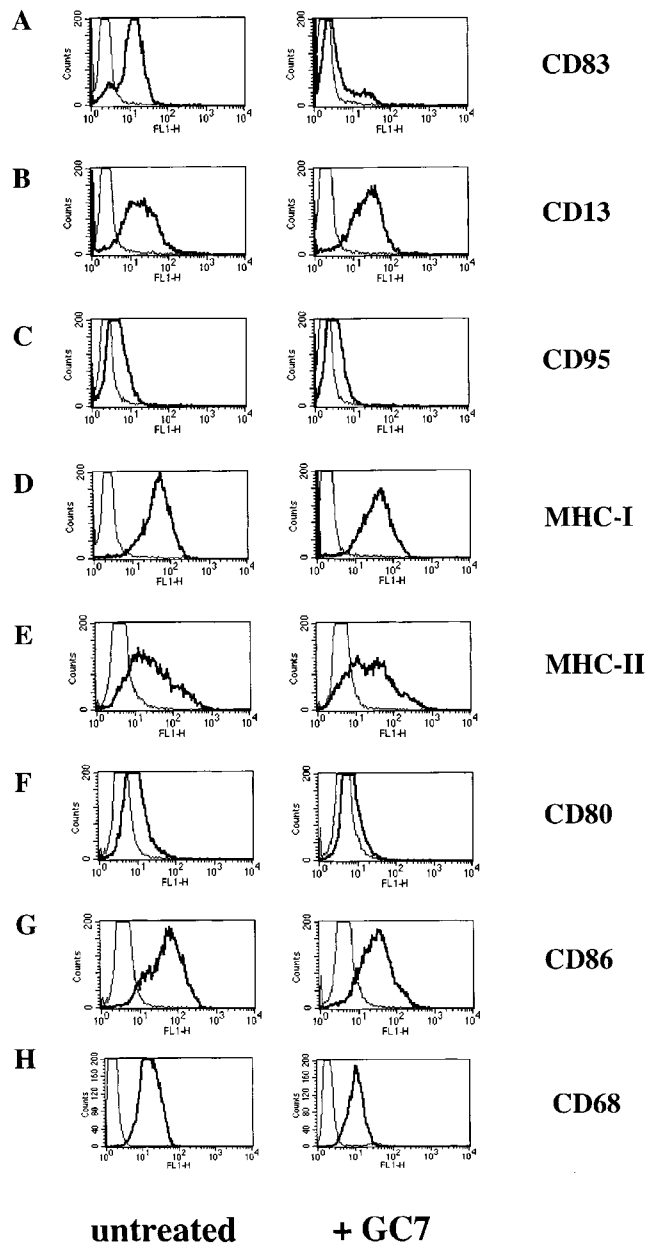


**Figure 1.** Detection of eIF-5A and CD83 protein expression during in vitro generation of human DCs by Western blot analysis. Cellular protein extracts from different DC generation time points (indicated at the bottom) were resolved by SDS-PAGE, transferred to nylon membranes, and probed with (A) CD83-specific, (B) eIF-5A-specific, or (C) histone H1-specific (loading control) antibodies.



**Figure 2.** Effect of GC7 on CD83 cell surface expression. Human DC precursors were subjected to CD83-specific indirect immunofluorescence analysis. (A) No significant CD83 surface expression was detectable in immature DCs (indicated by asterisks) at day 5. (B) Analysis of mature cells at day 7 demonstrated a strong CD83 surface expression. (C) Comparable signals were detected when DMSO was present in the cell cultures. (D) Exposure of precursor cells to the hypusine inhibitor GC7 (1  $\mu$ M in DMSO) demonstrated a significant inhibitory effect on CD83 cell surface expression. (E) Quantification of the CD83-specific fluorescence signals shown in A–D.

reflecting the data obtained in the experiments using total cell protein extracts (Fig. 1 A). In sharp contrast, when GC7 was added to the cell culture medium on day 5 (1  $\mu$ M), a strong decrease in CD83-specific immunofluorescence was observed at the end of the maturation period (day 7, Fig. 2 D). A control experiment demonstrated that DMSO (solvent for GC7) alone had no significant inhibitory effect on CD83 surface expression (Fig. 2 C). Quantification of the fluorescence signals (Fig. 2 E) confirmed that the presence of the hypusine modification inhibitor GC7 indeed reduced the surface expression of CD83 at least fivefold during the *in vitro* generation of human DCs.



**Figure 3.** FACS® analyses of untreated or GC7-treated DCs. Left panels show the typical phenotype of untreated mature DCs. Right panels show the effect of GC7 treatment. (A) GC7 strongly reduced CD83 cell surface expression. No significant GC7 effect on cell surface expression was observed in case of CD13 (B), CD95 (C), MHC class I and II molecules (D and E, respectively), and CD68 (H). Only a minimal reduction was observed for CD80 and CD86 cell surface expression (F and G, respectively).

For further characterization of the effect of GC7 on DC precursors, we next extended our studies with GC7 to other cell surface molecules expressed on mature DCs. FACS® analyses confirmed that the cell surface expression of CD83 was significantly reduced (Fig. 3 A). In contrast, CD95 (Fig. 3 C), a molecule involved in programmed cell death (41), the MHC class I and II molecules (Fig. 3, D and E), CD13 (Fig. 3 B), which is a protein constitutively ex-

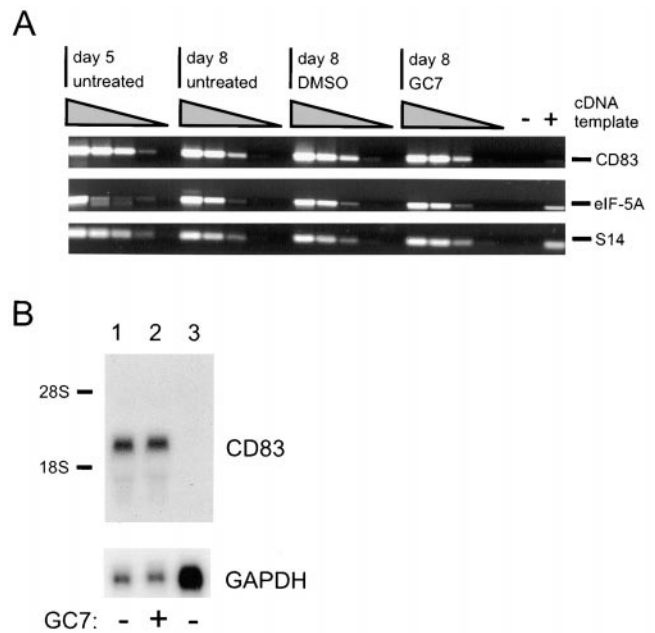
pressed on myeloid cells (42), and CD68 (Fig. 3 H), a molecule that discriminates between DCs and macrophages (43), were not affected by GC7 treatment. Furthermore, expression of the costimulatory molecules CD80 and CD86 was only slightly reduced (Fig. 3, F and G). Importantly, when fully mature DCs were treated with GC7, no alteration in the expression of the cell surface molecules analyzed above was observed, indicating that only immature DC precursors are sensitive to GC7. Finally, GC7 did not induce necrosis or apoptosis as determined by propidium iodide and annexin V staining (data not shown).

Taken together, the data presented demonstrate that the biosynthesis of the hypusine-containing protein eIF-5A is specifically regulated during DC maturation and that hypusine formation appears to be required for surface expression of the DC marker molecule CD83.

**GC7 Affects the Intracellular Distribution of CD83-specific mRNA in DCs.** To obtain an insight into how GC7 interferes with CD83 expression, we next prepared total cellular RNA from immature and mature DCs that were cultured in the presence or the absence of GC7. Subsequently, these RNAs were reverse transcribed into single-stranded cDNAs, which were then analyzed by PCR using specific oligonucleotide primer pairs. Amplification of ribosomal protein S14 RNA-derived sequences served as an internal control, and serial 10-fold dilutions of the input templates (cDNAs) indicated linear PCR kinetics. The relative levels of the investigated mRNAs were comparable, irrespective of whether or not GC7 or the solvent control DMSO was present in the cell cultures (Fig. 4 A). This highly sensitive RNA assay also revealed that CD83-specific mRNA is already detectable on day 5 of the DC maturation period and, most importantly, that GC7 treatment did not alter the CD83 mRNA levels. We next subjected total cellular RNA isolated from untreated or GC7-treated (days 5–8) DCs to CD83-specific Northern blot analysis (44), thereby demonstrating again that GC7 does not have a significant influence on the overall CD83 mRNA level in DCs (Fig. 4 B, lanes 1 and 2). As expected, no CD83 messages were present in total RNA derived from HeLa cells (Fig. 4 B, lane 3). Taken together, these data suggested that CD83 surface expression is blocked by GC7 at the posttranscriptional level.

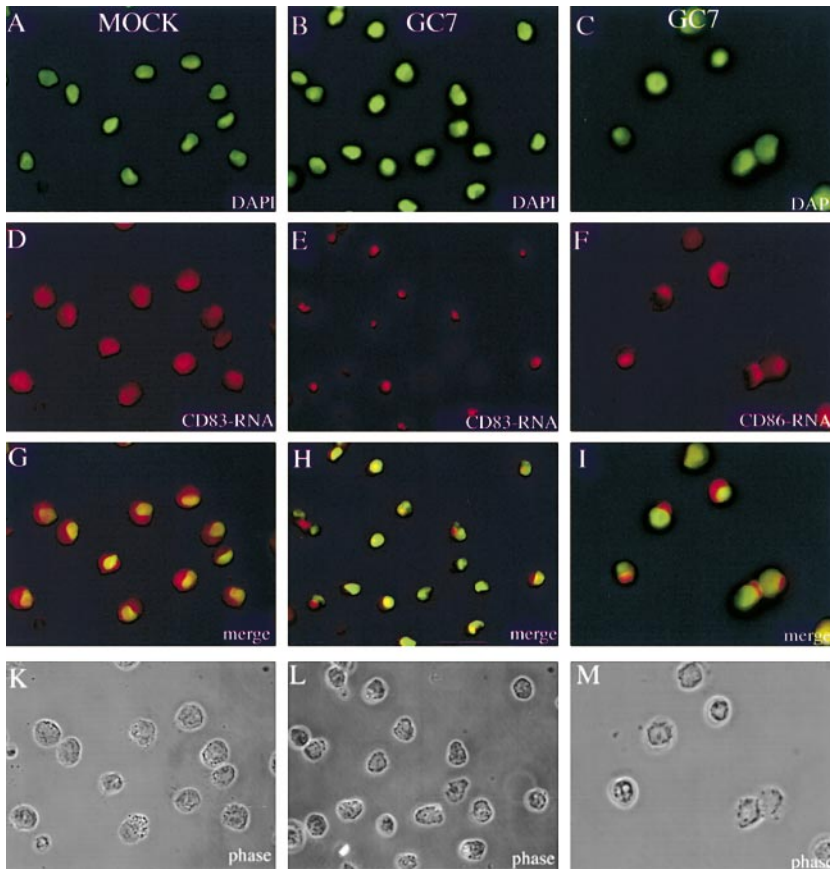
As the hypusine-containing protein eIF-5A has been shown to be involved in the nucleocytoplasmic transport of HIV-1- and HTLV-I-specific mRNAs (21–24), it is conceivable that GC7 may affect the intracellular distribution of CD83-specific mRNA. This would mean that eIF-5A activity, which critically depends on the posttranslational hypusine modification of the eIF-5A precursor, mediates efficient nuclear export and thereby, indirectly, the translation of CD83 mRNA.

To test this hypothesis, we next directly examined potential GC7-dependent accumulation of CD83 mRNA in the cytoplasmic and nuclear compartment of DCs. As only limited amounts of DCs can be obtained from blood samples, a direct biochemical fractionation of total cellular



**Figure 4.** Detection of eIF-5A- and CD83-specific mRNAs. (A) Total cellular RNA was isolated from untreated, DMSO-treated, and GC7-treated DC precursors at the indicated time points and reverse transcribed. Using specific oligonucleotide primers, serial dilutions of the respective cDNAs were subjected to PCR and subsequently analyzed by agarose gel electrophoresis. Amplification of ribosomal protein S14 RNA-specific sequences served as control for input cDNA amounts. The identities of the various amplification products (indicated at right) were confirmed by DNA sequence determination (not shown). –, negative PCR control; +, positive PCR control. (B) Northern blot analysis of total cellular RNA isolated from DCs that were matured in either the absence (lane 1) or presence (lane 2) of GC7. CD83-specific mRNA species were detected, irrespective of whether or not GC7 was present in the cell cultures. In contrast, no CD83 transcripts were detected in total RNA derived from HeLa cells (lane 3). To control for loading of comparable quantities of RNA, the filters were stripped and rehybridized using a probe specific for GAPDH mRNA.

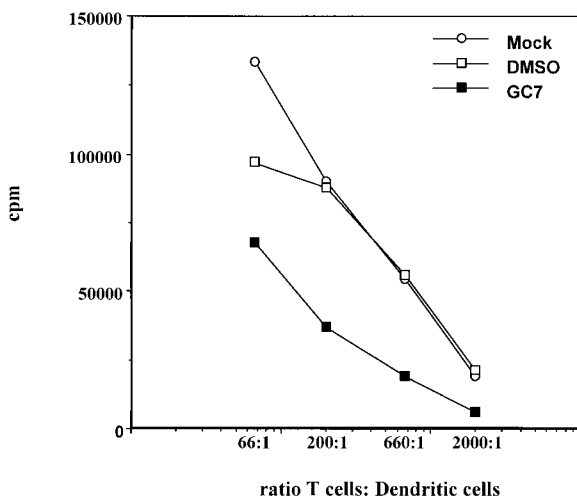
RNA in nuclear and cytoplasmic subfractions was not feasible. We therefore subjected our *in vitro*-generated DCs to CD83 mRNA *in situ* hybridization. The nuclei of fixed DCs (Fig. 5, K, L, and M) were visualized by DNA staining using DAPI (Fig. 5, A, B, and C). To prevent the loss of cells through nonadherence in this type of experiment, special adhesion slides were used that affect the typical DC morphology. Inspection of the CD83-specific signals demonstrated that almost comparable levels of CD83 mRNA accumulate in the DC nucleus and cytoplasm (Fig. 5 D). However, in the presence of GC7, the CD83 mRNA appears to be trapped in the nuclear compartment (Fig. 5 E). This becomes particularly obvious when the respective images are merged (compare Fig. 5, G and H). Furthermore, it appears that the CD83 mRNA is not randomly distributed within the nuclei of GC7-treated cells but tends to accumulate at distinct subnuclear sites. This GC7 effect is specific for CD83 mRNAs, since addition of GC7 did not result in nuclear trapping of CD86-specific messages (Fig. 5



**Figure 5.** Subcellular localization of CD83 and CD86 mRNA in DC precursor cells. Images belonging to the same experiment are aligned in columns. Mock-treated (CD83: A, D, G, and K) or GC7-treated (CD83: B, E, H, and L; CD86: C, F, I, and M) DC precursors were subjected to CD83 mRNA- or CD86 mRNA-specific in situ hybridization. Nuclei were labeled by DNA staining using DAPI (A, B, and C). mRNAs were visualized with digoxigenin-labeled oligonucleotide probes, followed by primary  $\alpha$ -digoxigenin and appropriate secondary Cy3-coupled antibodies (D, E, and F). Comparison of the merged images shows equal distribution of CD83 mRNA between the nucleus and the cytoplasm in mock-treated DCs (G). In contrast, GC7 treatment of DCs results in nuclear accumulation of CD83 mRNA (H). As shown in panel I, GC7 treatment of DCs does not result in nuclear trapping of CD86 mRNA. Corresponding phase contrast images are shown in K, L, and M.

F). Please note that these data are in good agreement with the FACS<sup>®</sup> analyses shown in Fig. 3 G.

These data suggest that CD83 mRNA is transported from the DC nucleus to the cytoplasm by exploitation of a



**Figure 6.** Effect of GC7 on the ability of DCs to induce allogeneic T cell proliferation. Mature DCs derived from untreated (○) or DMSO-treated (□) precursors induce a strong allostimulatory reaction in the primary allogeneic MLR. In contrast, cells derived from GC7-treated precursors demonstrate a reduced allostimulatory capacity (■).

specific pathway. Moreover, interference with this pathway results in nuclear trapping of CD83 mRNA and therefore in decreased CD83 protein synthesis.

*GC7 Inhibits the Ability of DCs to Induce Allogeneic T Cell Proliferation.* The most distinctive functional characteristic of DCs is their ability to induce a potent T cell response (45). Therefore, we studied whether or not the observed GC7 effect on DC surface molecules, and in particular the prominent inhibition of CD83, also affected the ability of DCs to induce T cell proliferation in an allogeneic MLR. As depicted in Fig. 6, mock- or DMSO-treated DCs displayed comparable T cell proliferation induction rates in this experiment. Clearly, GC7 impaired the ability of DCs to induce a significant T cell response in the MLR. Although these experiments do not show that CD83 downregulation alone is responsible for reduced stimulation, the data indicate that hypusine formation is required for DC function.

## Discussion

Functional, mature DCs are derived from circulating precursor cells after a period of maturation (4, 46). During this period, many activities at the level of gene regulation take place within the precursor cells which ultimately result in formation of mature DCs (47). In addition to their functional qualities, DCs are also characterized by the expression of a specific array of marker molecules. These include

the accessory/costimulatory gene products CD40, CD80, and CD86 as well as MHC class I and II (1, 3). In particular, the presence of the CD83 molecule is a well characterized marker for fully mature DCs, as CD83 can not be detected on immature DC precursors (37). The functional significance of CD83 is, however, completely unknown.

In this study, we show that CD83 expression is upregulated during DC maturation and is closely mirrored by the expression pattern detected for the hypusine-containing protein eIF-5A. More importantly, our experiments show that GC7, a highly potent inhibitor of the unique hypusine modification in eIF-5A, significantly inhibits CD83 surface expression in these cells. As previously published data have demonstrated that eIF-5A plays a functional role in HIV-1 Rev- and HTLV-I Rex-mediated nuclear export of retroviral mRNAs (21–24), it was tempting to speculate that GC7 may also interfere with the translocation of the CD83 mRNA across the nuclear envelope in DCs. The notion of a potential GC7 effect on intracellular CD83 mRNA transport was further supported by the recent finding that eIF-5A binds the export receptor CRM1 and accumulates at nuclear pore-associated intranuclear filaments, the site where initial docking of export substrates to the nuclear pore complex is believed to occur (29). Thus, the inhibition of nucleocytoplasmic translocation of CD83 mRNA by GC7 should indeed result in the observed downregulation of CD83 cell surface expression. In fact, examination of the intracellular distribution of CD83 mRNA in our precursor cells demonstrated a marked redistribution of these transcripts between the subcellular compartments upon addition of GC7. GC7 clearly prevented efficient cytoplasmic accumulation of CD83 mRNA, as the messages remained trapped in the cell nucleus, an effect that was not observed for CD86 mRNA. Taken together, these data suggest that hypusine formation is required for efficient nuclear export of CD83 mRNA. Thus, CD83 mRNA exploits a specific pathway for its transport from the nuclear site of RNA transcription and processing to the site of translation in the cytoplasm.

Competition experiments in *Xenopus* oocytes suggested that different classes of RNA (e.g., mRNA, rRNA, U-rich snRNA, and tRNA) are exported from the nucleus by specific export factors (48). General nuclear export of mRNA appears to be mediated by a class of abundant RNA binding proteins termed heterogeneous nuclear ribonucleoproteins (hnRNPs; for review see references 49 and 50). hnRNPs associate with poly(A)<sup>+</sup> RNA in the nucleus as well as in the cytoplasm, and some subsets of hnRNPs have been shown to shuttle between these cellular compartments (51). However, little is known about the regulation of nuclear export of specific groups of mRNAs. A series of recent studies in which the metabolism of early response gene (ERG) mRNA subsets was investigated provided new evidence that specifically regulated mRNA export pathways may indeed exist in mammalian cells (for review see reference 52). ERG mRNAs encode functionally important proteins such as protooncogenes, cytokines, and lymphokines that are characterized by a short half-life due to

instability sequences, termed AU-rich elements (AREs). ARE sequences are commonly found in the 3'-untranslated region of these short-lived mRNAs (53). However, binding of the HuR (also called HuA) nucleocytoplasmic shuttling protein to ARE sequences significantly increases the stability of ERG mRNAs (54–57) and may subsequently lead to elevated ERG mRNA levels in the cytoplasm. Thus, differences in mRNA nuclear export rates can have pronounced effects on the expression of specific genes, providing cells with an effective mechanism to quickly respond to environmental signals. The data presented in this study also suggest that CD83 mRNA, which does not contain obvious ARE-rich regions (44), can accumulate in the cytoplasm by virtue of a similar mRNA transport mechanism during DC differentiation. Such a mechanism would assure the timely and efficient expression of critical DC proteins. The existence of such a mechanism would indeed be beneficial in the case of CD83. As a result of the specific surface expression on mature DCs only, CD83 is considered to be an important protein for DC function, although its precise activity in DCs remains elusive.

Clearly, the inhibitory effect of GC7 on DC activity can not be attributed exclusively to the lack of CD83 expression. We cannot rule out the possibility that GC7 may also affect the metabolism of additional mRNAs in DCs, which have not yet been identified. Nevertheless, interference with eIF-5A function provides a new method to investigate DC function at the molecular level that may ultimately result in the identification of additional functionally important DC proteins. Furthermore, the pharmacological interference with hypusine formation may also provide a novel approach to modulate DC activity.

As DCs are able to induce both immunity and tolerance, they represent a very promising cell type for future immune therapy studies. The elucidation of the mode of action of DC-specific molecules, like CD83 on the molecular level, will not only be important to understand the biology of DC but also be advantageous for the development of new therapeutic strategies.

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