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DC-SCRIPT Regulates Glucocorticoid Receptor Function and Expression of Its Target GILZ in Dendritic Cells

Saatje Hontelez, Nina Karthaus, Maaike W. Looman, Marleen Ansem, and Gosse J. Adema

Dendritic cell (DC) maturation occurs upon pathogen recognition and inflammation in the peripheral tissue and involves upregulation of costimulatory molecules and inflammatory cytokine secretion. Fully matured DCs migrate to T cell areas in the lymph nodes, inducing T cell activation (1). In addition, DCs can also be activated to become tolerogenic, suppressing inflammation. Both human and murine studies demonstrated that this process involves impaired DC activation, which can be triggered by ligand-dependent activation of the glucocorticoid receptor (GR) prior to pathogen recognition (2). Within DCs, GR activation impairs STAT, NF-κB, AP-1, 14-3-3, Raf-1, and Ras signaling, thereby preventing upregulation of costimulatory molecules upon DC maturation. Expression of the GR target gene glucocorticoid-inducible leucine zipper (GILZ) is induced upon glucocorticoid (GC) stimulation and serves an important role in mediating the immunosuppressive effects by GR. In fact, expression of GILZ in the absence of GR activation was demonstrated to be sufficient for the generation of tolDCs, whereas silencing GILZ expression prevented tolerance (3). GC-treated DCs displayed suppressed MHC I-restricted Ag presentation (4, 5), impaired upregulation of costimulatory molecules (6, 7), and reduced secretion of proinflammatory cytokines. Secretion of immunosuppressive cytokines was enhanced in these cells (7–9). Collectively, these effects result in altered DC-mediated T cell activation, with reduced Th1 responses and selective expansion of T regulatory cells (10, 11). This does not only apply to monocyte-derived DCs (moDCs) but also myeloid DCs (mDCs) (12) and plasmacytoid DCs (pDCs) (13) have been shown to become tolerogenic following GC exposure.

The immunosuppressive effects of GCs are typically mediated by GR, a type I nuclear receptor (NR). The ligand-free form of GR predominantly resides in the cytoplasm, complexed to chaperone proteins. Conformational changes through ligand binding release GR from the chaperone complex, allowing for nuclear translocation and transcription initiation (14).

The human GR is encoded by a single gene and is expressed in virtually all cell types. Various GR isoforms have been described, and tissue-specific effects are currently attributed to variation in GR isoform expression (15–17). GR pre-mRNA can be alternatively spliced generating the transcriptional active GRα and the repressor GRβ, differing in sequence only at the C terminus ligand binding domain. In contrast to GRα, GRβ expression is confined to the nucleus, where it antagonizes GRα-dependent gene transcription (18). In addition, translation reinitiation occurs at seven AUG-start sites at the mRNA 5′-end, generating eight different GR polypeptides. These isoforms, termed GR-A, -B, -C1, -C2, -C3, -D1, -D2, and -D3, differ in length at the N terminus and in glucocorticoid responsiveness, differentially affecting target gene expression. The GR-A, -B, and -C isoforms are localized in the cytoplasm, translocating to the nucleus upon ligand binding where

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Abbreviations used in this article: CLSM, confocal laser scanning microscopy; CBP1, C-terminal binding protein 1; DC, dendritic cell; DC-SCRIPT, dendritic cell–specific transcript; dexamethasone; GC, glucocorticoid; GILZ, glucocorticoid-inducible leucine zipper; GR, glucocorticoid receptor; HA, hemagglutinin; HCK293, human embryonic kidney 293; HRE, hormone-responsive element; iDC, immature dendritic cell; IP, immunoprecipitation; MDC, myeloid dendritic cell; mDC, mature dendritic cell; MMTV, mouse mammary tumor virus; moDCs, monocyte-derived dendritic cell; NR, nuclear receptor; Oct1, octamer-binding transcription factor-1; PBGD, porphobilinogen deaminase; PDC, plasmacytoid dendritic cell; pred, prednisolone; RR1, retinoid X receptor; sILRNA, small interfering RNA; siSC, DC-SCRIPT targeting siRNA; tolDC, tolerogenic dendritic cell.
they initiate target gene transcription. In contrast, GR-D isoforms are, independent of ligand binding, confined to the nucleus and have markedly lower transcriptional activity (16).

Recently, we have identified DC-specific transcript (DC-SCRIPT) as a transcription factor and an NR coregulator in human breast and prostate carcinoma tissue. DC-SCRIPT was found to be present in type I and type II NR protein complexes, repressing type I NRs estrogen receptor-, progesterone receptor-, or androgen receptor-mediated transcription while enhancing type II NRs retinoic acid receptor α/retinoid X receptor (RXR), vitamin D receptor/RXR, or peroxisome proliferator activated receptor γ/RXR function (19–22).

In the immune system, DC-SCRIPT is specifically expressed in DCs and, in contrast to other DC markers, identifies all DC subsets tested to date (23, 24). Interestingly, we have demonstrated an important role for DC-SCRIPT in TLR4- and TLR7/8-mediated DC maturation. DC-SCRIPT knockdown in moDCs decreased secretion of the anti-inflammatory cytokine IL-10, which subsequently impaired both the production of the proinflammatory cytokine IL-12 as well as T cell proliferation (25). In this study, we investigated the putative role of DC-SCRIPT in controlling the anti-inflammatory function of GR in moDCs. Our data show the presence of DC-SCRIPT in GR protein complexes and demonstrate altered GR-mediated transcription in the presence and absence of DC-SCRIPT. Our findings therefore suggest active regulation of GR function by DC-SCRIPT in DCs.

**Materials and Methods**

**Cell lines**

Human embryonic kidney 293 (HEK293) cells were cultured in DMEM containing GlutaMAX (Invitrogen, Breda, The Netherlands) supplemented with 10% heat-inactivated FCS (Greiner Bio-one, Alphen a/d Rijn, The Netherlands), 1% nonessential amino acids (Invitrogen), and 0.5% antibiotic-antimycotic (Invitrogen). Human hepatocellular carcinoma Hep3B cells were cultured in IMDM (Invitrogen) supplemented with 10% heat-inactivated FCS and 0.5% antibiotic-antimycotic (Invitrogen).

**Generation of human DCs**

Human moDCs were generated from PBMCs as described previously (26). Monocytes were derived from buffy coats from healthy donors (Sanquin, Nijmegen, The Netherlands). Buffy coats were obtained from healthy volunteers after informed consent and according to institutional guidelines. Plastic-adherent monocytes were cultured for 6 d in DCs culture medium (phenol red-free RPMI 1640 medium (Life Technologies, Breda, The Netherlands) supplemented with 1% ultra-glutamine, 0.5% sodium deoxycholate) containing the protease inhibitors 2 μg/ml leupeptin (Sigma-Aldrich), 2 μg/ml aprotinin (Roche), and 1 mM PMSF (Cellgenix). During day 3 moDCs were supplemented with new IL-4 (300 U/ml) and GM-CSF (450 U/ml). Mature moDCs were generated from day 6 immature moDCs through 24-h stimulation with vehicle (0.1% EtOH), following 24 h in immunoprecipitation (IP) assay buffer (50 mM NaCl, 1% Triton X-100, 0.1% NaDodSO₄, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 0.5% sodium deoxycholate) containing the protease inhibitors 2 μg/ml leupeptin (Sigma-Aldrich), 2 μg/ml aprotinin (Roche), and 1 mM PMSF (Cellgenix). Cell lysates were used for IP of DC-SCRIPT using anti–DC-SCRIPT antibody or actin and GR were visualized on the same blot using two different secondary antibodies conjugated with fluorescent molecules detected at 800 and 680 nm. After staining, the membranes were scanned using the Odyssey Infrared Imaging system to visualize the labeled proteins.

**Transfection**

HEK293 cells were seeded in 10-cm culture dish (6 × 10⁶ cells/dish) 24 h prior to transfection. Cells were cotransfected with 3 μg pCAT-DC-SCRIPT or pCATCH (control) and 5 μg pHA-n3/GRA or pHα-n3 (control) by using Metafectene transfection reagent (3 μg/ml, 1% ultra-glutamine, 0.5% antibiotic-antimycotic (Invitrogen)), according to the manufacturer’s protocol. Cells were lysed 24 h after transfection in immunoprecipitation (IP) assay buffer (50 mM NaCl, 1% Triton X-100, 0.1% NaDodSO₄, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 0.5% sodium deoxycholate) containing the protease inhibitors 2 μg/ml leupeptin (Sigma-Aldrich), 2 μg/ml aprotinin (Roche), and 1 mM PMSF (Cellgenix). Cell lysates were used for IP of DC-SCRIPT using anti–DC-SCRIPT–coupled dynabeads (GE Healthcare, Hoekelaren, The Netherlands), according to the manufacturer’s protocol. Isootype-coupled Dyna-Beads were used as a control.

**Western blotting**

Cells were subjected to electrophoresis on an 8% 37:5:1 acryl/bisacrylamide gel and transferred onto Protran nitrocellulose transfer membrane (Macherey-Nagel). moDCs were harvested at 100 × 10⁶ cells/ml (3 μl, 1:80; Abcam) and IRDye 680CW donkey–anti-mouse IgG (1:1000; Li-cor Biosciences) or IRDye 680CW goat–anti-rabbit IgG (1:5000; Li-cor Biosciences) as secondary Ab. GR was detected with a rat anti-HA (1:1000, 3F10; Roche) and IRDye 800CW goat-anti-rat IgG (1:5000; Li-cor Biosciences) or anti-GR Ab [3D5] (200 μg/ml; 1:80; Abcam) and IRDye 680CW goat–anti-mouse IgG (1:5000 Li-cor Biosciences) as secondary Ab. β-actin was detected with a mouse anti-β-actin (1:20000; Roche Applied Science) or rabbit-anti-actin (Sigma-Aldrich) and IRDye 680CW donkey–anti-mouse IgG or goat–anti-rabbit (1:5000; Li-cor Biosciences) as secondary Ab, as loading control. Both actin and DC-SCRIPT or actin and GR were visualized on the same blot using two different secondary detection Abs conjugated with fluorescent molecules detected at 800 and 680 nm. After staining, the membranes were scanned using the Odyssey Infrared Imaging system to visualize the labeled proteins.

**Luciferase transfection assay**

Hep3B cells were plated (6 × 10⁵) in 24-wells plates 8 h before transfection and transfected using the Calcium Phosphate precipitation method (Invitrogen). HEK293 cells were plated at (1 × 10⁵) in 24-well plates 24 h before transfection and transfected using metafectene. Transfected cells were stimulated with 100 nM dexamethasone (Sigma-Aldrich) in 1% E/B/3% BSA in PBS with 0.01% Tween. DC-SCRIPT was detected with anti–DC-SCRIPT (1.2 μg/ml; R&D Systems) and IRDye 680CW donkey–anti-goat IgG (1:5000; Li-cor Biosciences) as secondary Ab. GR was detected with a rat anti-HA (1:1000, 3F10; Roche) and IRDye 800CW goat-anti-rat IgG (1:5000; Li-cor Biosciences) or anti-GR Ab [3D5] (200 μg/ml; 1:80; Abcam) and IRDye 680CW goat–anti-mouse IgG (1:5000 Li-cor Biosciences) as secondary Ab. FLUOROSCAN was detected with a mouse anti-β-actin (1:20000; Roche Applied Science) or rabbit-anti-actin (Sigma-Aldrich) and IRDye 680CW donkey–anti-mouse IgG or goat–anti-rabbit (1:5000; Li-cor Biosciences) as secondary Ab, as loading control. Both actin and DC-SCRIPT or actin and GR were visualized on the same blot using two different secondary detection Abs conjugated with fluorescent molecules detected at 800 and 680 nm. After staining, the membranes were scanned using the Odyssey Infrared Imaging system to visualize the labeled proteins.

**DC-SCRIPT knockdown**

Human moDCs day 4 were electroporated with a 23-nt Custom ZNF366 small interfering RNA (siRNA) termed DC-SCRIPT targeting siRNA.
(siSC) targeting the DC-SCRIPT gene at position 2349–2369 (Dharmacon, Lafayette, CO) or the irrelevant siRNA ON-TARGETplus Non-Targeting siRNA#1 (Dharmacon) termed control. Cells were washed twice in PBS and once in OptiMEM without phenol red (Invitrogen). A total of 10 μg siRNA was transferred to a 4-mm cuvette (Bio-Rad), and 10 × 10^4 DCs were added in 200 μl OptiMEM and incubated for 3 min before being pulsed with an exponential decay pulse at 300 V, 150 μF in a GenePulse Xcell (Bio-Rad) as described previously (27). Immediately after electroporation, the cells were transferred to warm (37°C) DC culture medium without antibiotic/antimycotic and supplemented with 1% ultra-glutamine, 0.5% antibiotic–antimycotic, 10% (v/v) FCS, IL-4 (300 U/ml), and GM-CSF (450 U/ml). Day 6 (72 h after transfection) DCs were stimulated with vehicle (0.1% EtOH) or 100 nM RU-486 (Sigma-Aldrich) for 1 h and subsequently with vehicle (0.1% EtOH) or 100 nM pred for 24 h. RNA was isolated with the Quick-RNA MiniPrep kit (ZymoResearch). Total lysates were prepared 72 h after transfection, lysing 50,000 cells in 50 μl 1% SDS lysis buffer containing 1% SDS and 62.5 mM Tris (pH 6.8) plus the protease inhibitors 2 μg/ml leupeptin, 2 μg/ml aprotinin, and 1 nM PMSF.

RNA isolation and quantitative PCR
Total RNA was isolated from cells using an RNA isolation kit (ZymoResearch). RNA quantity and purity were determined on a NanoDrop spectrophotometer. Total RNA was DNase-I (amplification grade; Invitrogen) treated, and redissolved using water. cDNA was synthesized using random hexamers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). mRNA levels for the genes of interest were determined with a CFX96 sequence detection system (Bio-Rad, Veenendaal, The Netherlands) with SYBR Green (Roche) as the fluorophore and gene-specific oligonucleotide primers. The primers for DC-SCRIPT and porphobilinogen deaminase (PBGD) were described previously (20). Other used primers (forward, reverse): GR (5'-CCATGTCGACGAAGGAAAGAACC-3', 5'-ATGGTTCAGCTATACATTCTCGG-3'), GILZ (5'-AGAACCTCAATCGACGAAAG-3', 5'-GTCATGATGTTCTTACCA-3'), Reaction mixtures and program conditions were used that were recommended by the manufacturer (Bio-Rad). Quantitative PCR data were analyzed with CFX Manager V1.6.541.1028 software (Bio-Rad) and checked for correct amplification and dissociation of the products. As a reference gene, the housekeeping gene porphobilinogen deaminase (PBGD) was used. DC-SCRIPT and GILZ levels relative to PBGD were calculated according to the cycle threshold method \[2^{-\Delta\Delta Ct_{DC-SCRIPT or GILZ - Ct_{PBGD}}}\] (28). Differences in mRNA expression were assessed using t tests. Two-sided p < 0.05 was a priori for samples to be considered statistically significant.

**Results**

**DC-SCRIPT coimmunoprecipitates GR**

DC-SCRIPT was previously shown to be present in protein complexes containing type I and/or type II NRs using co-IP assays (20). Whether DC-SCRIPT is also present in GR containing protein complexes is unknown. To investigate this, whole-cell lysates were prepared from HEK293 cells cotransfected with expression vectors encoding DC-SCRIPT and GR or their controls. IPs were performed on these lysates using goat–anti-DC-SCRIPT–coated beads or control goat–IgG-coated beads. Both the immunoprecipitated fractions and the total lysates were subjected to immunoblotting. The data show an effective IP of DC-SCRIPT using goat–anti-DC-SCRIPT Ab–coated beads, whereas unspecific binding to the control beads is minimal (Fig. 1). In addition, GR was effectively coimmunoprecipitated with goat–anti-DC-SCRIPT–coated beads from lysates of HEK-293 cells that were cotransfected with DC-SCRIPT and GR. No GR co-IP was observed when DC-SCRIPT was cotransfected with control hemagglutinin (HA) only. These data demonstrate that in transfected HEK293 cells, DC-SCRIPT and GR can exist in the same protein complex. This occurs most likely through indirect interaction, because the co-IP of GR with DC-SCRIPT could only be demonstrated when using mild lysis conditions (data not shown).

**DC-SCRIPT represses GR function on MMTV**

Next, we investigated whether DC-SCRIPT affects transcriptional activity of GR by using luciferase reporter assays. Hep3B cells were cotransfected with DC-SCRIPT, GR, or their controls and a reporter construct containing the MMTV promoter controlling luciferase expression (MMTV-luc). In the MMTV promoter, four hormone-responsive elements (HRE) have been identified that bind ligand-activated GR, thereby inducing transcription of the luciferase gene (29). Eighteen hours after transfection, the cells were treated with vehicle or the GR ligands dexamethasone (dex) or pred. Luciferase production was analyzed 24 h after stimulation.

Hep3B cells lack endogenous GR expression; therefore, only background luciferase activity was detected after GR–ligand stimulation in the absence of ectopically expressed GR. Cells that were cotransfected with the GR expression vector did show luciferase activity in a GR ligand–dependent manner. Both dex and pred were able to induce luciferase production (Fig. 2A). Interestingly, increasing expression levels of DC-SCRIPT resulted in a dose- and ligand-dependent repression of GR activity. Previously, DC-SCRIPT has been shown to directly interact with the global transcription corepressor C-terminal binding protein 1 (CtBP1) via its CtBP1 interaction motif (24). In addition, DC-SCRIPT harbors an LxxXL motif, a motif known to facilitate interactions with NRs (5). Next, we examined whether the CtBP1 and the NR binding (LxxXL) motifs within DC-SCRIPT were important for its repressive function on GR. We therefore mutated both CtBP1 domains or deleted the LxxXL motif. Both mutants were tested for protein expression levels (data not shown), which resembled the expression levels of wild-type DC-SCRIPT. Cellular localization was also expected to be similar, because deletion of the complete acidic domain does not alter its nuclear location (24). Fig. 2B shows that both mutants are still able to strongly repress GR-dependent luciferase production. Thus, DC-SCRIPT functions as a corepressor of GR-induced transcription on the MMTV promoter, independent of its CtBP1 binding and LxxXL motif.

Efficient transcription initiation via the MMTV promoter requires the presence of the most distal HRE site (HRE1), the regulatory element AA upstream of HRE1 (30) and binding of the transcription factors NF-I and octamer-binding transcription factor-1 (Oct1) (29, 31, 32). To assess the requirement of these regulatory elements for DC-SCRIPT–mediated corepression, we deleted these sequence elements from the MMTV-luc reporter. In the MMTVΔ1-luc and MMTVΔ2-luc reporters, the AA element alone or both the HRE1 and the AA element are deleted, respectively, whereas the MMTVΔNF-I/Oct1-luc reporter lacks the NF-I and Oct1 binding sites (Fig. 2C) (33). The wild-type MMTV-luc reporter was used as positive control. Deletion of the AA element or the NF-I/Oct1 binding sites reduced GR-dependent luciferase expression compared with the wild-type reporter, which was even
further decreased upon deletion of both AA and HRE1 (Fig. 2D).

On all MMTV reporters/mutants, DC-SCRIPT expression effectively repressed luciferase production. These data imply that the repressive function of DC-SCRIPT does not depend on the HRE1 and the AA element nor does the NF-I and Oct1 binding sites.

DC-SCRIPT represses transcriptional activity of GR isoforms

GR has been shown to be translated into eight different isoforms using alternative translational start sites (Fig. 3A) (17). The GR expression vector includes all translational start sites, thus, could express all GR isoforms. Indeed transfection of GR in HEK293 cells demonstrates expression of the full-length GR-A, as well as the GR-B, -C, and, albeit at markedly lower levels, GR-D isoforms (Fig. 3A). To examine DC-SCRIPT function on different GR isoforms, we also cloned GR-C3 and GR-D3. Of note, the GR-C3 vector includes downstream ATGs, hence, in addition to GR-C3, this vector could theoretically also express GR-D1, -D2, and -D3. However, only GR-C3 and GR-D1 could be detected (data not shown).

The effect of DC-SCRIPT on transcription initiation of the reporter construct MMTV-luc by these isoforms was tested using luciferase transcription assays. GR isoforms expressed by both GR and GR-C3 expression vectors equally induced luciferase production in a ligand-dependent manner. Cells expressing the GR-D3 isoform, however, were less potent in inducing luciferase expression, displaying a 4-fold reduction in luciferase production.

Increasing expression levels of DC-SCRIPT resulted in a dose- and ligand-dependent repression of the transcriptional activity of all GR isoforms (Fig. 3B). This indicates that the 1–336 aa N-terminal part of GR that was deleted in the GR-D3 expression vector is not required for the effect of DC-SCRIPT on GR-dependent transcription.

DC-SCRIPT and GR expression in immature, mature, and tolerogenic DCs

Next, we investigated DC-SCRIPT and GR protein expression by western blotting and CLSM in immature (iDCs), mature (mDCs) and tolerogenic DCs (tolDCs). iDCs and mDCs were obtained by

FIGURE 2. Effect of DC-SCRIPT on GR-mediated transcription. Luciferase reporter assay for GR-mediated transcription. Hep3B cells were cotransfected with the firefly luciferase reporter plasmid MMTV-luc, the expression plasmid for GR and increasing amounts of the expression plasmid for wild-type DC-SCRIPT (A) or with mutated CtBP- or deleted LxxLL binding sites (B). Cells were stimulated with vehicle, 100 nM dex, or 100 nM pred for 24 h. Luciferase activity is displayed relative to luciferase production upon dex stimulation in the presence of GR and absence of DC-SCRIPT. (C) Schematic representation of firefly luciferase reporters. Reporters consist of the MMTV promoter with four HRE in front of a TATA-box controlling luciferase gene expression. Three MMTV mutants have been created from the wild-type (MMTVwt), deleting the upstream 3′-end including the AA domain (MMTVΔ1), the 3′-end and the most distal HRE (MMTVΔ2), or the binding sites for NF1 (NF-I) and Oct1 (MMTVΔNF-I/Oct1). (D) Luciferase reporter assay for GR-mediated transcription. Hep3B cells were cotransfected with the firefly luciferase reporter plasmids MMTVwt-luc, MMTVΔ1, MMTVΔ2, or MMTVΔNF-I/Oct1, the expression plasmid for GR, and increasing amounts of the expression plasmid for DC-SCRIPT. Cells were stimulated with vehicle (N) or 100 nM dex (n) for 24 h. Luciferase activity is displayed relative to luciferase production upon dex stimulation in the presence of MMTVwt and in the absence of DC-SCRIPT. Data from at least three independent experiments. Error bars correspond to ± SEM.
stimulating immature moDCs with vehicle or 24 h of LPS, respectively. tolDCs were generated from immature moDCs through 24-h stimulation with dexamethasone and subsequent 24 h with LPS. Western blot analysis of DC-SCRIPT and GR protein levels showed equal expression of both proteins in iDCs, mDCs and tolDCs (Fig. 4A). The translational isoforms GR-A, -B, -C, and -D isoforms visualized with rat-anti-HA in GR-HA–transfected HEK293 cells. (B) Luciferase reporter assay for GR-A, -C, and -D–mediated transcription. Hep3B cells were cotransfected with the firefly luciferase reporter plasmid MMTV-luc, expression plasmids for GR-A, -C, or GR-D and increasing amounts of the expression plasmid for DC-SCRIPT. Cells were stimulated with vehicle (○) or 100 nM dex (●) for 24 h. Luciferase activity is displayed relative to luciferase production upon dex stimulation in the presence of GR-A and in the absence of DC-SCRIPT. Data from at least three independent experiments. Error bars correspond to ± SEM.

Knockdown of DC-SCRIPT enhances GILZ expression in moDCs

To determine the physiological relevance of the interaction between DC-SCRIPT and GR, DC-SCRIPT expression was silenced in moDCs using siRNA electroporation. Because DC-SCRIPT was demonstrated to repress GR-mediated transcription on the MMTV promoter, we hypothesized a similar effect on the endogenous GR target GILZ in moDCs. DC-SCRIPT knockdown was therefore expected to enhance GR-dependent transcription, which could be detected by an increase in GILZ expression.

Human moDCs were electroporated at day 4 with a siSC or an irrelevant control siRNA oligo. DC-SCRIPT protein expression was markedly reduced in siSC-treated DCs 48 and 72 h after electroporation (Fig. 5A), whereas GR expression remained unaltered (Fig. 5B). At day 6, moDCs were treated with vehicle or the GR antagonist RU-486 for 1 h and subsequently stimulated with vehicle or the GR agonist prednisolone for 24 h. After stimulation, GILZ mRNA levels were measured. As expected, little or no GILZ mRNA expression was detected in vehicle-treated control DCs. Stimulation with pred resulted in an upregulation of GILZ mRNA.
expression levels in control DCs (Fig. 5C), which could be effectively blocked by inhibiting GR activation with the GR antagonist RU-486. Strikingly, vehicle-treated siSC DCs already demonstrated a significant (p = 0.012) increase in GILZ expression compared with control DCs. This upregulation appears to be GR independent, because it is also detected in the presence of the GR antagonist RU-486. In the absence of the GR antagonist, treatment with pred further increased GILZ expression in DC-SCRIPT knockdown DCs, with relative GILZ mRNA levels being significantly higher than in control siRNA-treated DCs (p = 0.01). These data thus indicate DC-SCRIPT to function as corepressor of GR-mediated transcription of GILZ in DCs. Blocking GR activation with the GR antagonist abolished GILZ upregulation in DC-SCRIPT knockdown DCs, confirming that this was indeed dependent on GR activation. These results indicate that DC-SCRIPT affects GR activity in DCs and represses expression of the endogenous GR target gene GILZ, a transcription factor that has been associated with the generation of tolDCs.

**Discussion**

The present study demonstrates that the NR coregulator DC-SCRIPT modulates GR function in human moDCs. In cell lines, DC-SCRIPT was present in GR-containing protein complexes, and GR-mediated transcription was found to be repressed by DC-SCRIPT. Human moDCs coexpress DC-SCRIPT and GR in the nucleus and cytoplasm, and DC-SCRIPT silencing resulted in enhanced expression of the GR target gene GILZ, a transcription factor that is instrumental for the generation of tolDCs.

Copresence of DC-SCRIPT and GR in protein complexes could be demonstrated in transfected HEK293 cells; however, we could not co-IP the endogenous complex from DCs. This could have several reasons. Human monocyte derived DCs express at least 20 different NRs of which at least 6 (GR, progesterone receptor, estrogen receptor, retinoic acid receptor α, vitamin D receptor, and peroxisome proliferator activated receptor γ) possibly contain DC-SCRIPT in their repressor or activator complexes (19–22). Therefore, enrichment of a specific NR via DC-SCRIPT IP may be extremely difficult. Furthermore, endogenous DC-SCRIPT not present in protein complexes may possibly bind more efficiently to the beads, and is therefore more likely to be purified. In addition, DC-SCRIPT and GR likely interact indirectly. Hence, lysis buffers need to be mild to keep the complex intact but at the same time sufficiently stringent to properly lyse the nucleus. Nonetheless, our co-IP findings in HEK293 cells are further supported by functional data that demonstrate the effect of DC-SCRIPT expression on GR–ligand-mediated GR target gene expression, not only in HEK293 cells, but also in dendritic cells.

The present data describing the interaction between DC-SCRIPT and GR in cell lines is in line with our previous findings, suggesting that DC-SCRIPT interacts with NRs via other proteins present in NR protein complexes. DC-SCRIPT has been shown to bind the transcription corepressors receptor-interacting protein 140 and CtBP1, as well as histone deacetylase-1, -3, and -6, all known to be present in NR protein complexes (5, 33, 34). The absence of a direct contact between DC-SCRIPT and GR is also consistent with the finding that deletion of the NR interaction motif LxxLL and the CtBP1 interaction motif in the acidic domain of DC-SCRIPT did not affect its repressive function on GR transcription. This implies that interaction with CtBP1 and the presence of the LxxLL motif is not required for the repressive effect of DC-SCRIPT. Interaction with NR complexes could, however, be facilitated via other, less defined NR interaction motifs (35–39). In addition to the LxxLL motif, DC-SCRIPT contains multiple putative NR interaction motifs that are possibly involved in the interaction with various NRs (data not shown).

Coexpression of DC-SCRIPT and GR in DCs was demonstrated with CLSM, albeit areas containing either DC-SCRIPT or GR were equally abundant. Ligand-dependent GR translocation was observed in iDCs. However, in the absence of its ligand, GR was also detected in the nucleus of iDCs. The nuclear GR expression most likely reflects the expression of GRβ isoforms, known to have restricted nuclear localization (18). DC-SCRIPT expression was
Because we have recently identified DC-SCRIPT as a NR coregulator, the human NR superfamily contains 48 members, of which 20 NRs are affected by differentiation, maturation, or both. The biology, affecting either differentiation, maturation, or both. The immunosuppressive effect of GR can be reproduced in the absence of GR ligand by IL-10 secretion and T cell proliferation, suggesting that DC-SCRIPT knockdown skews DCs toward tolerance.

In addition to GILZ, we also investigated the expression of other genes known to be induced by ligand dependent GR activation in other cells. However, identification of these other GR targets in DCs and whether they are affected by DC-SCRIPT knockdown proved to be difficult (data not shown). For 8 of 10 tested target genes no GR–ligand-dependent upregulation in moDCs could be detected. This could be due to cell-type specific differences between DCs and other cells like differences in the expression and function of the repressive GRα-D and GRβ isoforms. Increased expression upon GR activation was only detected for FKBP5 (51 kDa FK506-binding protein 5) and PTX3 (pentraxin 3); however, induction of DC-SCRIPT might differ depending on GR conformation and structural context (data not shown). As soon as a suitable linear motif is found to be produced in large amounts by tolDCs (42).

In this study, DC-SCRIPT knockdown in immature moDCs significantly increased IL-10 expression levels upon TLR4 and 7/8-mediated maturation. The cell surface markers CD80, CD83, and CD86 (data not shown), which show differential expression between mature DCs and tolDCs, were not affected by DC-SCRIPT knockdown and resembled levels found on mature DCs. Nonetheless, elevated IL-10 levels subsequently impaired IL-12 secretion and T cell proliferation, suggesting that DC-SCRIPT knockdown skews DCs toward tolerance.

In addition to IL-10, we also investigated the expression of other genes known to be induced by ligand dependent GR activation in other cells. However, identification of these other GR targets in DCs and whether they are affected by DC-SCRIPT knockdown proved to be difficult (data not shown). For 8 of 10 tested target genes no GR–ligand-dependent upregulation in moDCs could be detected. This could be due to cell-type specific differences between DCs and other cells like differences in the expression and function of the repressive GRα-D and GRβ isoforms. Increased expression upon GR activation was only detected for FKBP5 (51 kDa FK506-binding protein 5) and PTX3 (pentraxin 3); however, induction levels were low and DC-SCRIPT silencing did not affect expression of these genes (data not shown). One major reason could be the HRE sequence or the context of this sequence in the promoter of these genes. Meijsing et al. (43) demonstrated that a single nucleotide change in the GRE sequence influences GR binding affinity and conformation, affecting cofactor recruitment and transcription activation. Similarly, the binding properties of DC-SCRIPT might differ depending on GR conformation and affect the binding of other cofactors. Furthermore, adjacent binding sites of other transcription factors can also affect GR-mediated transcription and possibly DC-SCRIPT function (19).

In line with this, we have preliminary data that suggest that DC-SCRIPT can also activate GR-dependent transcription, depending on the promoter context (data not shown). As soon as a suitable DC-SCRIPT Abs for chromatin IP become available, it would be extremely interesting to perform chromatin IP–sequencing studies and expression arrays to further investigate gene regulation specifically in DCs with respect to DC-SCRIPT and GR activation and expression.

Finally, besides GR, also other NRs are known to function in DC biology, affecting either differentiation, maturation, or both. The human NR superfamily contains 48 members, of which 20 NRs have been described to be expressed in monocyte-derived DCs (44). Because we have recently indentified DC-SCRIPT as a NR coregulator in breast (20) and prostate carcinoma (22), affecting both type I and type II NR function, it is tempting to speculate about a role for DC-SCRIPT as NR coregulator in DC biology. It would therefore be interesting to study target gene expression of various NRs after DC-SCRIPT knockdown in DCs.

Disclosures
The authors have no financial conflicts of interest.

References

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