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Identification and Characterization of DC-SCRIPT, a Novel Dendritic Cell-Expressed Member of the Zinc Finger Family of Transcriptional Regulators

Vassilis Triantis,* Dagmar Eleveld Trancikova,* Maaike W. G. Looman,* Franca C. Hartgers,† Richard A. J. Janssen,* and Gosse J. Adema2‡* 

Dendritic cells (DC)1 play a pivotal role in adaptive immunity, because they are the initiators of immune responses. DC are APC that capture Ags in the periphery, process them, and present their peptides in the context of MHC molecules to T cells (1). To activate naïve T cells, DC must be activated. Different stimuli such as bacterial or viral products (LPS and dsRNA) (2–4), cell-to-cell interactions (CD40-CD40L) (5), or soluble factors (TNF-α) (6) are able to activate DC, a process referred to as DC maturation. Upon maturation, DC undergo several functional and morphological changes, such as up-regulation of costimulatory molecules, enhanced peptide loading of MHC classes I and II, and dendrite formation, and migrate to T cell areas of secondary lymphoid tissue where they can launch immune responses (1).

DC are the most potent APC, and they are involved in immunity and tolerance (7). There are two main subsets described (type I IFN-producing plasmacytoid DC (PDC) and myeloid DC (MDC)) that originate from different hemopoietic lineages, but also a certain subset, Langerhans cells (LC), that resides exclusively at the skin. Distinct subsets may exert diverse functions, such as modulating the type of immune response (8, 9). Even though their functional capacity has been well demonstrated, and DC have already been used in clinical trials, the molecular mechanisms that lie beneath their potency are still, to a great extent, unknown. There have been several DC-specific molecules described, and many of them relate to their immunological capacity, such as DC-specific ICAM-3 grabbing nonintegrin (DCSIGN), DC-chemokine 1, DC-specific transmembrane protein (DC-STAMP), and langerin (10–13), yet the molecular basis for their development and unique function remains largely unknown. Surely, DC induction of immune responses or tolerance must be fine-tuned, and a network of transcription factors has been implicated in DC development and immunobiology, including PU.1, SpiB, Id2, and RelB (14–17). For example, SpiB promotes plasmacytoid DC development while blocking T, B, and NK cell development from hemopoietic precursors. Cross-talk between C/EBP transcription factors and PU.1 is required for myeloid DC development and differentiation. PU.1 is expressed in multiple hemopoietic lineages as well as CD34+ cells, and PU.1-null mice were unable to generate MHC class IIhighCD11c+ MDC in vitro. Id2 is induced by TGF-β and was also shown to orchestrate LC development by repressing B cell genes in DC, whereas RelB, a component of the NF-kB complex of transcription factors, is a critical regulator of DC differentiation. In mice, the lack of RelB impairs DC derived from bone marrow in both number and function. Thus, a balanced network of transcription factors governs the development and function of DC.

To characterize more genes entangled in DC immunobiology, we previously applied differential display PCR (DD-PCR) to DC. We report the identification of a novel putative transcription repressor expressed in DC, DC-SCRIPT. DC-SCRIPT encodes for a unique protein with a putative DNA binding domain flanked by domains that could be involved in gene regulation. The gene is expressed by several DC subsets, both in vitro and in vivo,
suggesting an important function for the protein in the differentiation pathway of DC.

Materials and Methods
Leukocyte preparations
PBMC were obtained by leukapheresis of healthy donors, and adherence for 2 h resulted in a nonadherent PBL fraction. Monocytes were eluted from PBMC by counterflow centrifugation and were stimulated with 2 μg/ml LPS for 16 h. DC were generated in vitro from adherent monocytes as described previously (18). Purified tonsil B lymphocytes were isolated as described previously (19). Blood DC were isolated from PBMC using the MACS Blood DC isolation kit (CLB). Mature CD11c+ (MDC) blood DC were obtained by culture in RPMI 1640 (In Vitrogen Life Technologies) enriched with 10% FCS and 50% (v/v) MCM for 3 days. CD4+CD11c+ (PDC) blood DC were obtained by an additional immunomagnetic depletion of CD11c+ cells with microbeads (Dynal Biotech) and were matured in RPMI 1640 medium enriched with 10% FCS and 100 U/ml IL-3 (San- doz) for 3 days, followed by 1 μg/ml CD40L for a additional 24 h. LC were isolated as cells that had migrated out of epidermal sheets derived from healthy donors undergoing plastic surgery of breast or abdomen (42 h) in the presence or absence of 500 U/ml GM-CSF (Scherer-Plough). LC were enriched using anti-HLA-DR mAbs and MACS (CLB) and were >98% pure as analyzed by FACS.

DD-PCR
DD-PCR was performed as previously described (20). The 3′ primers used were the anchored oligo(dT) primers T1gMC, T3gMA, T7gMT, and T2gMG, which M represents A, C, G, or T. The 5′ primers were randomly designed oligonucleotides of 10 bases. PCR was performed in the presence of [35S]dATP to allow visualization of the products after separation by denaturing PAGE. PCR products that were reproducibly cell specific were enriched using anti-HLA-DR mAbs and MACS (CLB) and were isolated as cells that had migrated out of epidermal sheets derived from healthy donors undergoing plastic surgery of breast or abdomen (42 h) in the presence or absence of 500 U/ml GM-CSF (Scherer-Plough). LC were enriched using anti-HLA-DR mAbs and MACS (CLB) and were >98% pure as analyzed by FACS.

cDNA library screenings
cDNA libraries were prepared as described and screened using the randomly labeled 155-bp DC-SCRIPT fragment from the differential display PCR as a probe (primers, 5′-CCCTGCTATCTGTTGACA-3′ and 5′-TTCGGAAGAATACACAGT-3′). The most 3′ end of the DC-SCRIPT cDNA was isolated by preparing a cDNA library with a DC-SCRIPT-specific primer (5′-GCCGACGCGGCGCTCGTTATTCACT-3′). The most 5′ end of the DC-SCRIPT cDNA library was also isolated by preparing a cDNA library with a DC-SCRIPT-specific primer (5′-CTCGAGCTTTCAGATAATCAGTT-3′) and 5′-CTCGAGAAGAATACACAGT-3′) and randomly labeled with 35P (T7 QuickPrimer Kit; Pharmacia Biotech).

RT-PCR
Total RNA was transcribed into cDNA using an oligo(dT) primer and SuperScript II reverse transcriptase (In Vitrogen Life Technologies). Primers for DC-SCRIPT were located in the original DD-PCR product, yielding a specific product of 144 bp (24 cycles; 5′-ACGGTTAGACTAACTGACAGCAGA-3′ and 5′-CCTAGGACGGCCTGAAATCTCAGTT-3′). As a control for RNA quality, β-actin was amplified (18 cycles; 328 bp; forward and reverse primers, 5′-GAAAGAGTGAGCAGACACG-3′ and 5′-TCTGGGGTGTCCGAGTAGACG-3′). The cDNA was cloned into pGEM-T (Promega) as a fusion construct of DC-SCRIPT was derived from cloning the full-length ORF into the pcDNA4/TO/myc-His A vector (In Vitrogen Life Technologies). Site-directed mutagenesis was performed with the QuickChange Site-Directed Mutagenesis kit (Stratagene), and the mutants were subcloned into the pG8B9 (EcoRI-BamHI) and pEYFP-C1 (BglII-XhoI) vectors. Full-length C-terminal binding protein 1 (CtBP1) was inserted into the pFLAG-CMV-2 (Sigma-Aldrich) vector as a HindIII-Sall insert.

Yeast two-hybrid system
A yeast two-hybrid system was performed as described previously (25). Briefly, the acidic region of DC-SCRIPT was cloned into pG7BD (BD Clontech) as an EcoRI-BamHI insert. A DC-derived cDNA library was inserted in pGAD-GH (BD Clontech) in the EcoRI-Sall sites. Bait and prey plasmids were transformed into 1 M sorbitol, 10 mM bicine, and 3% ethylene glycol into yeast strain YGHI. Protein-protein interactions were reported by yeast growth on medium without leucine, tryptophan, or histidine. The expression of β-galactosidase was induced by β-mercaptoethanol and expression of β-galactosidase was induced by β-mercaptoethanol. Yeast colonies after replica filter lifting, N2 snap-freezing and incubation for 2–4 h in Z-buffer (60 mM NaHPO4, 60 mM Na2HPO4, 10 mM KCl, and 1 mM MgSO4) containing 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

Immunoblotting, immunoprecipitations, confocal microscopy, and transactivation assays
Whole-cell lysates were prepared in 1% SDS standard lysis buffer. Equal amounts of protein were separated by SDS-PAGE electrophoresis, and proteins were transferred to a Protran nitrocellulose transfer membrane (Schleicher & Schuell). The following primary Abs were used; mouse anti-β3-integrin mAb, ZEBRA, and clone BZ1 (DakoCytonama) in combination with a second HRP-conjugated goat anti-mouse IgG (H+L) Ab (0.4 μg/ml; Pierce). Immunoprecipitation was performed in a standard IP buffer with 1% Triton X-4% using protein G beads. For immunofluorescent staining, 2H3 HEK cells or DC were seeded on eight-chamber slides (Nunc) coated with fibronectin (20 μg/ml; Roche) or poly-L-lysine. Cells were fixed with methanol/aceton (1:1) and blocked with 3% BSA (Calbiochem) in PBS. The following Abs were used: M2 mouse anti-FLAG (Sigma-Aldrich), anti-β3 integrin mAb TS2/16, and mouse E-12-12 anti-CtBP (Santa Cruz Biotechnology). As isotype controls, IgG2a and IgG1 mAbs (BD Biosciences) were used. As secondary Ab, Cy5-conjugated goat anti-mouse IgG (H+L; Jackson ImmunoResearch Laboratories) and FITC- or Texas Red-conjugated goat anti-mouse IgG (Applied Biosystems). The DC-SCRIPT-specific probe was labeled at the 5′ end with a FAM fluorescent group and at the 3′ end with a TAMRA quencher group. The primers used yield a specific product of 104 bp and surround intron 4, resulting in a product of >3 kb on genomic DNA. The amount of DC-SCRIPT expression was normalized to the housekeeping gene GAPDH and compared with the expression of another housekeeping gene, porphobilinogen deaminase within the same donor. Calculations were performed as described by PerkinElmer.
Results
Differential display PCR identifies a novel DC-specific cDNA

Previously, we applied DD-PCR to identify novel transcripts that are specifically expressed by DC. Immature and mature monocyte-derived DC originating from two healthy donors were compared with a mixture of three monocytic, B, and T cell lines. The full-length cDNA corresponding to the 155-bp initial DD-PCR clone 203 was analyzed in further detail. As shown in Fig. 1A, clone 203 was distinctively present in DC, but not in the monocytes, B, or T cell lines. To confirm DC-specific expression of clone 203, RT-PCR with primers located within this 155-bp fragment was performed on an extensive panel of leukocyte populations and non-leukocytic cell lines (Fig. 1B). The analysis confirmed the preferential expression by DC and revealed that the adherent fraction of PBMC, which mainly consists of monocytes, expressed very low, but detectable, levels of the messenger. However, this might also be due to contamination with peripheral blood DC. Interestingly, the results demonstrated that clone 203 is preferentially expressed by DC. Therefore, we named the clone DC-SCRIPT (DC-Specific tranSCRIPT).

DC-SCRIPT gene encodes for an 8-kb messenger

Northern blot analysis with several different probes derived from the initial DC-SCRIPT cDNA clone identified a dominant RNA transcript of ~8 kb (Fig. 1C). The 8-kb RNA species was detected in both immature and mature DC (Fig. 1C, lanes 3 and 4), but not in PBMC (lane 5) or activated monocytes with LPS (lane 6). The nonadherent fraction (PBL) did not express any mRNA for DC-SCRIPT. The premonocytic cell line U937 and the T cell line Jurkat also did not express DC-SCRIPT mRNA (lanes 1 and 2, respectively). Tissue blot analysis showed a low level of expression in various tissues, including spleen, kidney, liver, heart, and placenta (data not shown). Possibly, such expression levels can be explained by residual DC in these tissues. Conclusively, DC-SCRIPT is found in DC, but not in other blood cell populations, resting or activated, confirming its preferential expression by DC.

DC-SCRIPT cDNA encodes a novel C2H2 zinc finger motif-containing protein

To obtain the full-length mRNA of DC-SCRIPT, we screened a DC cDNA library using the original 155-bp DD-PCR product as a probe. This resulted in the isolation of a 1.2-kb cDNA without an apparent ORF.

We next generated a DC cDNA library applying a specific primer resiling at the 5' end of the 1.2-kb DC-SCRIPT cDNA. Several screening procedures of this library finally resulted in the isolation of a cDNA clone of ~3700 nt. Sequence analysis of this clone revealed the presence of a single 2232-nt-long ORF, starting with the first ATG codon at nt 448, which is in the appropriate sequence context for translation initiation. The protein encoded by this ORF consisted of a proline-rich domain (aa 111–219), followed by 11 C2H2 zinc finger motifs (aa 255–556) and an acidic stretch of 103 residues (aa 561–663). Figure 2A illustrates the ORF and the domain structure. The C2H2 zinc fingers of DC-SCRIPT belong to the classical Cys-Cys-His-His-His subfamily of zinc fingers, as found in FOG-1 (friend of GATA1), transcription factor IIIA, and many other transcription factors. Zinc fingers can mediate protein-DNA, protein-RNA, or even protein-protein interactions in these transcription factors. The Cys-Cys-His-His motif, however, seems to be mainly involved in protein-DNA interactions (26, 27).

Database searches revealed that DC-SCRIPT is identical with the human gene of ZNF366 (RefSeq accession no. NM_152625.1) originally found to be homologous to the Fugu rubripes gene IZnfl1 (28). However, the homology with IZnfl1 is restricted solely to the zinc fingers region, which is 93% identical (Fig. 2B). Strikingly, outside the zinc fingers, DC-SCRIPT shares little or no homology with any other given protein, including transcription factors, underlining its unique identity within the family of zinc finger proteins.

Expression pattern of DC-SCRIPT in DC subsets

Using real-time semiquantitative PCR analysis, the expression of DC-SCRIPT in monocyte-derived DC was analyzed in further detail. Upon differentiation into immature DC with GM-CSF and...
IL-4, DC-SCRIPT is constitutively expressed from days 3–8 (data not shown). Stimuli such as CD40L, either alone or in combination with IFN-γ, did not have a significant effect on the expression level of DC-SCRIPT, whereas another DC-specific gene, DC-STAMP, was down-regulated under these conditions (Fig. 3A). The expression level of DC-SCRIPT was comparable to that of the housekeeping gene PBGD (Fig. 3A).

To investigate the expression of DC-SCRIPT by DC subsets in vivo, we isolated peripheral blood DC. Blood DC mainly consist of two defined subsets, CD11c⁺ MDC and CD11c⁺ PDC. Freshly isolated blood DC as well as cultured and CD40-activated PDC and MDC clearly expressed DC-SCRIPT, as shown in Fig. 3B. DC-STAMP was not expressed in freshly isolated blood DC and was only up-regulated during activation of the MDC, not in activated PDC. LC isolated from epidermal skin layers, were also positive for DC-SCRIPT, with or without the addition of GM-CSF (Fig. 3C). LC did not express DC-STAMP, illustrating once more that DC-SCRIPT is a novel marker expressed by all DC subsets tested to date.

Analysis of DC-SCRIPT protein

To characterize the DC-SCRIPT protein, we generated constructs encoding N- or C-terminal-tagged DC-SCRIPT fusion proteins: FLAG-DC-SCRIPT, DC-SCRIPT-GFP, and DC-SCRIPT-Myc-His. 293 HEK cells were transiently transfected with these constructs, and lysates were analyzed by SDS-PAGE and Western blotting using mAbs directed against the different tags. DC-SCRIPT is estimated to be ~75 kDa. In all cases, however, a specific protein band was detected of a somewhat larger size than the calculated size of the tagged DC-SCRIPT constructs (Fig. 4). Additional analysis demonstrated that the FLAG-DC-SCRIPT fusion protein migrated at the same position in the gel under both denaturing and nondenaturing conditions (data not shown). Possibly the size difference can be explained by the irregular electrophoretic mobility of the acidic C-terminal part of DC-SCRIPT. The electric charge of this region could affect its migration during SDS-PAGE. Alternatively, the difference in size could be attributed to some type of protein modification.
monocyte-derived DC (plus CD40L or CD40L and IFN-γ and LC. The expression of DC-SCRIPT in immature (day 6) and mature monocyte-derived DC (plus CD40L or CD40L and IFN-γ), compared with PBGD and DC-STAMP. B, Freshly isolated blood DC (day 0), blood DC plus MCM (day 3), and CD11c⁺ blood DC plus IL-3/CD40L were studied. C, LC that have migrated out of epidermal sheets in the absence or the presence of GM-CSF. Each graph represents data from one representative donor of two or more.

**FIGURE 3.** Expression pattern of DC-SCRIPT. A, Quantitative expression of DC-SCRIPT by in vitro cultured DC, freshly isolated blood DC, and LC. The expression of DC-SCRIPT in immature (day 6) and mature monocyte-derived DC (plus CD40L or CD40L and IFN-γ), compared with PBGD and DC-STAMP. B, Freshly isolated blood DC (day 0), blood DC plus MCM (day 3), and CD11c⁺ blood DC plus IL-3/CD40L were studied. C, LC that have migrated out of epidermal sheets in the absence or the presence of GM-CSF. Each graph represents data from one representative donor of two or more.

DC-SCRIPT localizes to the nucleus

To investigate the localization of DC-SCRIPT, DC-SCRIPT-YFP and a series of DC-SCRIPT-YFP deletion mutants were constructed (Fig. 5A). These constructs were transfected into 293 HEK cells. Part of the cells were cultured on fibronectin-coated slides and analyzed by confocal laser scanning microscopy, whereas the remainder was used to make lysates and verify protein expression. All DC-SCRIPT-YFP deletion mutants were properly expressed at the protein level (Fig. 5A). Full-length DC-SCRIPT-YFP localized to the nucleus of cells, as shown by simultaneous propidium iodide staining of DNA (Fig. 5B). We noted that both full-length FLAG-DC-SCRIPT and DC-SCRIPT-Myc-His localized to the nucleus, indicating that the nuclear localization is not affected by the tag (data not shown). Interestingly, neither deletion of the N-terminal region containing the putative NLS nor of the C-terminal part of DC-SCRIPT affected its nuclear localization (Fig. 5B). The construct containing the zinc finger region alone could drive the molecule in the nucleus. YFP can spontaneously localize in the nucleus and thus influence the outcome of such experiments. Therefore, we analyzed the localizations of two additional FLAG-tagged constructs (zinc-acidic and acidic regions). Also in this setting, the zinc fingers-containing construct was localized in the nucleus, confirming that this motif can determine the localization of the protein (Fig. 5B).

Transcriptional activity of DC-SCRIPT

To assess any possible transcriptional activity of DC-SCRIPT, we used the ZEBRA transactivation system described previously (23, 24). This system is based on the EBV transcription factor Zebra (BZLF 1). Selected parts of DC-SCRIPT were cloned into a pZd vector as shown in Fig. 6A. Zd encodes for a mutant Zebra protein lacking the transactivation domain of Zebra, but retains the DNA binding and dimerization domain of the native protein. The reporter construct contained seven Zebra-responsive elements upstream of the E4 minimal promoter coupled to luciferase (Fig. 6A). Transfection efficiencies were calibrated by means of a Renilla promoterless construct that had basal activity. The native ZEBRA protein and a fusion protein of the transactivation domain of VP16 to Zd (not shown) were used as positive controls. Western blots were performed to verify expression of the constructs in 293 HEK cells (data not shown). Experiments were repeated in 293 HEK and THP-1 cells, but none of the constructs was able to induce luciferase expression in either 293 HEK or THP-1 cells (Fig. 6B). We also investigated whether lack of transactivation was dependent on cell activation. Therefore, we repeated the experiment in THP-1 cells using the full-length protein of DC-SCRIPT, and cells were activated with 100 ng/ml PMA. PMA is known to activate the NF-κB pathway (29), and we hypothesized that DC-SCRIPT could be a downstream target of such activation. However, DC-SCRIPT failed to activate transcription in this setting as well (Fig. 6B).
DC-SCRIPT binds CtBP1 in vivo

To confirm DC-SCRIPT binding to CtBP1 in a mammalian system, we performed coimmunoprecipitations in 293 HEK cells. 293 HEK cells were transfected with FLAG-CtBP1 and YFP-fusion DC-SCRIPT constructs (Fig. 7B). Transfection with YFP together with FLAG-CtBP1 was used as a control. Another negative control was another region of DC-SCRIPT that normally should not bind to CtBP1 (zinc fingers). The same mutations that were tested in the yeast two-hybrid system were examined in 293 HEK cells for their ability to bind CtBP1. The results demonstrated that DC-SCRIPT could specifically precipitate CtBP1. Moreover, when we mutated the first site, DC-SCRIPT could no longer immunoprecipitate CtBP1. However, altering the second site did not have any affect on the binding of CtBP1. Consequently, when both sites were mutated, then CtBP1 could not interact with DC-SCRIPT. In accordance with the yeast two-hybrid results, DC-SCRIPT can bind CtBP1 in 293 HEK cells, and the motif at positions 590–594 is responsible for bringing together the two proteins.

Discussion

DC are the instructors of adaptive immune responses by guiding naive T cells against Ags that were captured in the periphery. Today DC are used in clinical trials against various types of cancer (30), but as yet little is known regarding the molecular signature of a DC. To date, various markers with expression restricted to DC have been described with variable functions. DC-SIGN, a C-type lectin involved in pathogen recognition (31–34); DC-chemokine 1, a chemokine recruiting naive T cells to germinal centers (11); langerin, another type II lectin (13); and DC-STAMP, a multiple membrane-spanning receptor that resides in the endoplasmic reticulum with an as yet unknown function (35), are some of these markers. However, none of these markers is expressed by all types of DC. For example, DC-SIGN is also expressed by macrophages.
under certain conditions (34), and although DC-STAMP is absent from LC (Fig. 3A), langerin is restricted to these cells (13).

In this article, we describe a novel DC marker, DC-SCRIPT. DC-SCRIPT is preferentially expressed by DC among the leukocyte populations tested. Expression of the messenger is an early hallmark of DC differentiation from monocytes. These findings suggest that DC-SCRIPT is essential for DC function and immunobiology. DC-SCRIPT is present at the mRNA level in various DC subsets, with an enduring expression throughout the DC life cycle. During activation, DC-SCRIPT is not significantly down- or up-regulated regardless of the stimulus applied. Other crucial proteins engaged in numerous DC subset differentiation and development also portray the same expression pattern while retaining steady levels during activation. SpiB and PU.1, for example, seem to govern plasmacytoid DC and LC development, respectively, and their expression is kept stable upon activation (15, 36).

Apart from its distinct expression pattern, DC-SCRIPT bares 11 Cys:Cys:His:His Zn fingers, a proline-rich domain, and an acidic region. Therefore, it seems to represent a transcription factor, but beyond the zinc fingers, it shares no homology with proteins. The N-terminal part of the protein contains a classical NLS. Such sequences are described to be necessary for nuclear import, and the major types of NLSs fall into two categories. The first type consists of a single cluster of basic amino acids, for example, PKKRRKV in the SV40 large T Ag NLS (37). The second type is the bipartite NLS, composed of two clusters of basic amino acids separated by eight to 16 random residues, i.e., KRPATTTGQAKKKK as found in nucleoplasmin (38). DC-SCRIPT contains a typical NLS (RKRR), albeit this does not seem to be essential for translocation of the protein in the nucleus, because DC-SCRIPT lacking the NLS still localizes in the nucleus. Our results show that the zinc finger domain alone can facilitate nuclear import of the whole molecule (Fig. 5B). However, no NLS-like stretch is present in this region, suggesting that there must be another mechanism for targeting the protein into the nucleus. Indeed, it has been shown that proteins such as pancreatic transcription factor-1 are carried in the nucleus via protein-protein interactions, rather than using an inherent NLS. In such a scenario, the protein uses a “Trojan horse” that takes the complex in the nucleus (39). The DNA binding domain encompasses 11 zinc fingers of the classical Cys-Cys:His-His type. Zinc fingers come in many types and forms, serving different functions. They can be DNA binding mediators or binding platforms for other proteins, such as RING fingers (26), or even bind themselves to RNA (27). In addition, there are examples of proteins with NLSs embedded in their DNA binding domain, such as Wilms’ tumor 1, JAZ, mouse orphan receptor (TR2), and erythroid Kruppel-like factor/Kruppel-like factor 1 (37, 40, 41). In these cases, the tertiary structure of the zinc fingers or even residues

![FIGURE 6. Transactivation capacity of DC-SCRIPT. A, Schematic representation of the effector and reporter constructs used during the transactivation assays with the Zebra system. DB, DNA binding domain; DZ, dimerization domain; P, proline-rich region; Zn, zinc fingers; Ac, acidic region; ZB, Zebra-responsive element; TATA, E4 minimal promoter. Besides the constructs, the DC-SCRIPT amino acids fused to the ZEBRA deletion mutant are indicated. B, Effect of DC-SCRIPT on transcription in THP-1 and 293 HEK cells. Results represent fold times luciferase expression. The inactive deletion mutant of ZEBRA Zd has a value of 1. Results were calibrated by use of Renilla luciferase.](http://www.jimmunol.org/)

![FIGURE 7. DC-SCRIPT interacts with CtBP1. A, Yeast transformed with wild-type and deletion mutants of the acidic region together with CtBP1 (β-galactosidase assay). Ac, acidic region wild type; Ac/mut1, acidic region with the first binding motif mutated; Ac/mut2, acidic region with the second binding motif mutated; Ac/double mut, acidic region with both binding motifs mutated. B, 293 cells transfected with wild-type and deletion mutants of the acidic region together with CtBP1. CtBP1 interacts with DC-SCRIPT only if the first motif is intact, whereas the second motif does not influence the interaction of the two proteins.](http://www.jimmunol.org/)
within some of the zinc fingers are sufficient for nuclear localization of the protein. Once the protein is in the nucleus, the zinc fingers retain it within by anchoring the protein to other nuclear targets or DNA. Indeed, DC-SCRIPT has basic residues within its zinc fingers that together can act as a less well-defined NLS, similar to erythroid Kruppel-like factor/Kruppel-like factor 1, but it can be assumed as well that some of the zinc fingers are responsible for nuclear import and retention, whereas others are binding DNA or interact with other proteins, comparable to CCTC binding factor, another 11-zinc finger protein (42). Therefore, DC-SCRIPT may use different combinations of zinc fingers to exert different functions.

We also investigated whether DC-SCRIPT has an effect on transcriptional activation. However, no such effect could be observed. Therefore, we applied the yeast two-hybrid system to identify cooperating molecules that could hint about the role of DC-SCRIPT. One of the molecules produced was CtBP1. CtBP1 is a corepressor that recognizes the PXDLS motif as an anchoring site on other proteins (43). DC-SCRIPT bares two such sites in its acidic region, and by site-directed mutagenesis, we pinpointed the exact site of the interaction. CtBP1 cooperators can be DNA binding proteins that use CtBP1 as an intermediate to finally recruit histone deacetylases and block transcription of their binding locus (44–46). We have evidence that DC-SCRIPT is a DNA-binding protein, and cooperation with CtBP1 can represent a repression mechanism for DC-SCRIPT. That could explain the lack of transcriptional activation of the constructs tested in Fig. 6. Interestingly enough, CtBP1 has also been implicated in hemopiogenesis by interacting with members of the Ikaros family of proteins (47).

Taken together, our results show that DC-SCRIPT is the first marker for DC that has been found in all DC subsets tested to date and probably represents a transcriptional repressor. Nevertheless, its role in DC immunobiology remains evasive. Future studies including knockout mice are essential to answer this question.

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Disclosures
V. Triantis, M. W. G. Looman, and G. J. Adema are with the University Medical Center in Nijmegen, The Netherlands, and are among the inventors of a patent with the provisional title of “Dendritic cell polypeptide DC-SCRIPT and application in immune modulation”.

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