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Induction of CML28-specific cytotoxic T cell responses using co-transfected dendritic cells with CML28 DNA vaccine and SOCS1 small interfering RNA expression vector

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Abstract

CML28 is an attractive target for antigen-specific immunotherapy. SOCS1 represents an inhibitory control mechanism for DC antigen presentation and the magnitude of adaptive immunity. In this study, we evaluated the potential for inducing CML28-specific cytotoxic T lymphocytes (CTL) responses by dendritic cells (DCs)-based vaccination. We constructed a CML28 DNA vaccine and a SOCS1 siRNA vector and then cotransfect monocyte-derived DCs. Flow cytometry analysis showed gene silencing of SOCS1 resulted in higher expressions of costimulative moleculars in DCs. Mixed lymphocyte reaction (MLR) indicated downregulation of SOCS1 stronger capability to stimulate proliferation of responder cell in DCs. The CTL assay revealed transfected DCs effectively induced autologous CML28-specific CTL responses and the lytic activities induced by SOCS1-silenced DCs were significantly higher compared with those induced by SOCS1-expressing DCs. These results in our study indicates gene silencing of SOCS1 remarkably enhanced the cytotoxicity efficiency of CML28 DNA vaccine in DCs.

Keywords: CML28; Suppressors of cytokine signaling 1; Cytotoxic T lymphocytes; Dendritic cell; Small interfering RNA

Dendritic cells (DCs) are the most powerful antigen-presenting cells for initiating primary immune response *in vitro* and *in vivo* [1]. Given their central role in cell-mediated immunity *in vivo*, DC represents a model cell type for developing infectious and malignant disease vaccination studies. DC-based vaccination targeting tumor-associated antigen (TAA) is a feasible and promising means of delivering tumor-specific immunotherapy [2–4]. The efficiency of such tumor vaccines will depend largely upon the functional status of DCs for optimal stimulation of CTL-mediated anti-tumor immune responses [2–4].

The efficacy of antigen presentation and stimulatory capability of DCs are the most important challenges for DC vaccination [2,3]. DC maturation serves as the critical switch from the maintenance of self-tolerance to the induction of immunity [1]. The suppressors of cytokine signaling (SOCS) family includes at least eight members, each of which plays a unique role in attenuating cellular signaling. SOCS1 negatively regulates cytokine signaling through molecules such as interferon (IFN)-γ, interleukin (IL)-2, IL-6, IL-7, IL-12, and IL-15, by inhibiting the Janus kinase cascade and suppressing the signal transducer and activator of transcription (JAK/STAT) pathway [5,6]. Recent research revealed that the stimulatory capacity of DCs and the magnitude of adaptive immunity are regulated negatively by SOCS1 [5–7]. This evidence demonstrates that SOCS1 represents an inhibitory control mechanism for DC antigen presentation and the magnitude of adaptive immunity. To enhance antigen-specific anti-tumor immunity of DC-based vaccine, it would make sense to circumvent

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the critical constraint of SOCS1 on DCs function by siRNA silencing [6,7].

CML28 is a novel 28 kDa TAA identified by serological analysis of cDNA expression libraries (SEREX) screening of a chronic myeloid leukemia (CML) cDNA expression library [8]. As a potent target for tumor-specific immunotherapy, CML28 is superior to other known TAAs characterized by its broader spectrum expression in tumor cells and stricter expression in normal tissues [8,9]. As such, CML28 is overexpressed in tumor cells, such as leukemia, lung cancer, melanoma, and prostate cancer, but not in normal hematopoietic or other tissues, with the exception of testis. Furthermore, previous studies reported that CML28 is a graft-versus-leukemia (GVL) target antigen of donor lymphocyte infusion (DLI), which indicates that CML28 can serve as a promising target for tumor-specific immunotherapy [8,10]. We report here, for the first time, the induction of CML28-specific CTL responses by DCs co-transfected with CML28 DNA Vaccine and SOCS1 siRNA vector *in vitro*.

Materials and methods

Cell lines and patients. The following cell lines were obtained from the American type culture collection (ATCC): human acute monomyelocytic leukemic cell line U937, human chronic myelogenous leukemia cell line K562, and human myeloid leukemia cell line HL60. The HLA-matching donor/recipient sibling pair was selected for DCs generation, CTL induction (donor), and target cells obtaining (recipient). All cells were maintained in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal calf serum (FCS, Hyclone, USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Generation and immunophenotyping of DCs. DCs were generated from peripheral blood mononuclear cells (PBMCs), as described previously [11]. In brief, the PBMCs were isolated from the healthy donor via Ficoll/Paque density gradient centrifugation. The cells were plated in 6-well plates (Cellstar, Germany) at 1×10^7 cells/well in serum-free RPMI 1640 medium (Gibco, USA) in a final volume of 5 ml. After incubation for 2 h at 37 °C, nonadherent cells were removed for the subsequently mixed lymphocyte reaction (MLR) culture, and the adherent cells were cultured in 20% heat-inactivated FCS RPMI 1640 plus 1.05 mM sodium pyruvate (Amersco, USA) and 25 mM Hepes (Amersco, USA) supplemented with 100 ng/ml granulocyte–macrophage colony-stimulating factor (GM-CSF, Peprotech, USA), 60 ng/ml stem cell factor (SCF, Peprotech, USA), and 40 ng/ml interleukin-4 (IL-4, Peprotech, USA).

Immunophenotyping was performed by FACSCalibur (Becton–Dickinson, USA). The following monoclonal antibodies were used: CD83-FITC, CD80-PE, CD14-PE, and CD86-FITC (eBioscience, USA). Non-reactive isotype-matched antibodies (eBioscience, USA) were utilized as controls.

Construction of CML28 DNA vaccine. To obtain human CML28 cDNA fragment, we performed RT-PCR with the following primers: CML28-5P1 (5′-CG*GAATTC*TGCGGACGCCCAGGAGGATG-3′) and CML28-3P1 (5′-GC*TCTAGA*GAGTGGGTGGAGGCAATG-3′). The reaction conditions were 35 cycles of 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 1 min. The CML28 cDNA fragment was subcloned into the *Eco*R1 and *Xba*1 sites in the multi-clone site of pcDNA3.1HisA (kindly provided by Xiling PhD of Tongji Hospital). Subsequently, the coding sequence of His-CML28 fusion protein was obtained from pcDNA3.1-HisA/CML28 using PCR with the following primers: CML28-5P2 (5′-CG*CTCGAG*CGGAGCTTACCATGG-3′) and CML28-3P2 (5′-TCC*CCGC GG*GGAGAGTGGGTGGAGGCAATC-3′), and then cloned into the *Xho*I and *Sac*II sites of pIRES2-EGFP (BD, USA) to generate CML28 DNA vaccine pHis-CML28-IRES2-EGFP. We verified the presence of the insert, directional cloning, and size by DNA sequencing.

Design of siRNA and construction of siRNA expression vector. The selection of siRNA sequences was based upon web-based program (http://www.sirnawizard.com/) and optimized according to Brummelkamp et al. [12–14]. Briefly, a 21-nucleotide sequence (5′-ACTACCTGAGTTCC TTCCCCT-3′) within the coding region of human SOCS1 (GenBank Accession No. AF132440) was selected and analyzed by BLAST (http://www.ncbi.nlm.nih.gov/blast/) to ensure that there were no significant homologies with other genes. Two complementary oligonucleotides (sense, 5′-TCCC **ACTACCTGAGTTCCTTCCCCT** *TCAAGAG* **AGGGGAAGG AACTCAGGTAGT** TT -3′, and antisense, 5′-CAAAAA **ACTACCTGAG TTCCTTCCCCT** *CTCTTGA* **AGGGGAAGGAACTCAGGTAGT**-3′) were synthesized chemically. A scrambled SOCS1 siRNA (5′-GCATCCC TTCTGCTCTCCTA-3′) was used as a negative control, which shows no significant homology to known gene sequences confirmed by BLAST analysis. Annealed siRNA was ligated into the *Bbs*I sites of the psiRNA-hH1neo vector, and the plasmid was sequenced and amplified routinely.

Transfection of DCs. DCs were harvested after seven days of culture, washed twice with cold serum-free RMPI 1640 and resuspended to a final concentration of 2×10^7 cells/ml in specified electroporation buffer. Subsequently, 5 μg of each plasmid, pHisA-CML28-IRES2-EGFP and psiRNA-hH1-neo-SOCS1, was mixed with 0.1 ml of the cell suspension, transferred to a 2.0 mm precooled electroporation cuvette, and nucleofected with an Amaxa Nucleofector™ apparatus (Amaxa, Germany) using human dendritic cell Nucleofector™ kit as described [15–17]. Following electroporation, cells were heated to 37 °C by adding fresh complete medium at 37 °C and then incubated at 37 °C in a humidified atmosphere supplemented with 5% CO_2. As controls, DCs were transfected with pHisA-CML28-IRES2-EGFP or mutant of SOCS1 siRNA alone to evaluate the effect of SOCS1 silencing on DCs function or SOCS1 gene silencing efficacy of siRNA, respectively.

Validation of expression of vaccine and efficiency of gene silencing. DCs were harvested at 8, 24, and 48 h post-electroporation respectively, and lysed in lysis buffer. Fifty microgram of the protein sample was separated by 10% denaturing SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes. The membranes were blocked at 4 °C overnight with 5% nonfat milk in Tris-buffered saline with Tween 20 (TBS-T) buffer and then were incubated with primary antibody anti-His mAb (Novagen, USA) diluted 1:1000 with 5% nonfat milk in TBS-T for 60 min at room temperature with gentle agitation. The membranes were washed three times between each step with 10 min duration each with TBS-T buffer. The protein antibody complexes were detected by an HRP-conjugated secondary antibody (Santa Cruz) using the enhanced chemiluminescence system (Pierce US). EGFP expression in transfected DCs was monitored in FACSCalibur and analyzed using the CellQuest software.

To evaluate the efficiency of SOCS1 gene silencing, transfected DCs were collected at 8, 24, and 48 h post-electroporation for RT-PCR. The RT-PCR was performed using the following primers of SOCS1-5P (5′-CA GTCTCCACAGCAGCAGAGC-3′) and SOCS1-3P (5′-TCCCGAGGC CATCTTCACG-3′) for SOCS1 and GAPDH-5P (5′-GCTGGCGCTG AGTACGTCGT-3′) and GAPDH-3P (5′-TGGGTGTCGCTGTTG AAGTC-3′) for GAPDH as an internal control. Cycling conditions were as follows: 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 20 s, annealing at 61 °C for 45 s, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 5 min.

CFSE proliferation assay. To assess stimulatory capacity of transfected DCs and proliferation in the responder cells, we labeled the nonadherent fraction of the PBMCs with carboxyfluorescein diacetate succinimidyl ester (CFSE; a kind gift of Junfa Xu PhD of Tongji Medical College) as described [18]. Immediately before labeling, we thawed 5 mM CFSE stock in DMSO (Sigma, Germany) and diluted it to 10 μM in an equal volume of PBS/BSA for 10 min. The labeling process was quenched with an equal volume of heat-inactivated FCS. After 1 min, the CFSE-labeled cells were washed twice with PBS, recounted, and adjusted to a concentration of 2×10^6 cells/ml in the culture media.

The MLRs were performed in 96-well U-bottomed microtiter plates (Cellstar, Germany). For the CTL induction, we incubated 5×10^5 various DCs (untreated, CML28-transfected, and SOCS1-silenced) with 2.5×10^6 autologous PBMCs in RPMI1640 with 10% FCS, respectively. After three days in culture, the cells were restimulated with corresponding DCs and cultured with 4 ng/ml of human recombinant IL-2 (Peprotech, USA) on days one and three. FACS analysis was performed to evaluate the proliferative response of PBMCs on days one, three, and five. The cytolytic activity of the induced CTLs was analyzed by a standard ^{51}Cr-release assay.

CTL assay. The standard ^{51}Cr-release assay was performed as described previously [11]. Briefly, primary leukemia cells and CML28-transfected DCs were used as targets. As controls, autologous PBMCs, untreated DCs, and leukemia cell lines (U937, HL60, and K562) were used, respectively. The target cells were labeled with ^{51}Cr–sodium chromate in RPMI 1640 for 1 h at 37 °C. After three washes, the 5×10^4 ^{51}Cr-labeled targets and the effector cell serial dilutions at various E:T ratios were incubated in 200 μl of RPMI 1640 in 96-well U-bottomed plates. After 4 h of incubation at 37 °C, 50 μl of the supernatant was harvested, and the released ^{51}Cr was measured with a scintillation counter. The maximal release was determined by adding 1% Triton X-100. The percentage of the specific lysis was calculated as follows: 100 × (experimental release − spontaneous release/maximal release − spontaneous release).

Statistical analysis. For statistical analysis, we used Student's t test, and a 95% confidence limit was taken to be significant, defined as $P < 0.05$.

Results

Expression of CML28 DNA vaccine and efficacy of SOCS1 gene silencing

After seven days of culture, the cells displaying the morphological characterism of immature dendritic cells were harvested for subsequent DNA transfection. Transfection of DCs mediated by nucleofection yielding predominant transfection efficiency of 41–56% was assessed by FACS analysis (Fig. 1a). The efficiency of DC transfection was as high as 44% as early as 8 h after nucleofection, where it remained over the next 24 h. By 48 h post-nucleofection the transfection efficiency was still high, reaching 40%. Western blotting verified the expression of the His-CML28 fusion protein in both the transfected and co-transfected DCs at various time points post-electroporation, but no apparent expression was observed in untreated DCs (Fig. 1b).

To investigate SOCS1 regulation of DC functions, we first identified a siRNA that specifically downregulates SOCS1. As verified by semi-quantitative RT-PCR assays, the level of SOCS1 in the total DC population transfected with SOCS1 siRNA was specifically decreased by approximately 43–52%, compared with levels in DCs transfected with a SOCS1 siRNA mutant that cannot downregulate SOCS1.

SOCS1 silencing contributes greatly to DC maturation

After seven days in culture, the cells were harvested and characterized phenotypically validated by FACS analysis. The harvested cells exhibited the typical phenotype of immature DCs: CD14neg, CD80low, CD86low, and CD83neg (Fig. 2). CML28-transfected DCs showed a slight increase of CD83, CD80, and CD86, suggesting cDNA loading contributes to DCs maturation (Fig. 2) [19,20]. In contrast, the FACS analysis of surface expression of differentiation markers revealed that the SOCS1-silenced DCs retained the typical phenotype of mature DCs, including: CD14neg, CD80high, CD86high, and CD83high (Fig. 2). This finding demonstrates that SOCS1 silencing greatly promotes DC maturation.

Downregulation of SOCS1 in DCs greatly enhances proliferation of the responder cell

FACS analysis of the CFSE-MLR revealed the kinetics of proliferation of the autologous PBMCs in the MLR cultures. On day one, the responder cells primed by both CML28-transfected and SOCS1-silenced DCs have similar proliferation index (Fig. 3a, $P > 0.05$) and were stained uniformly with CFSE (Fig. 3b). By the third day, the responder cells primed by SOCS1-silenced DCs divided up to four rounds of mitosis (Fig. 3b) and with higher PI (Fig. 3a, $P < 0.01$), in contrast with the ones of untreated and CML28-transfected DCs. The gap was much greater on day five still characterized with more times of division (Fig. 3c) and higher PI of the responder cells (Fig. 3a, $P < 0.01$) in siRNA-treated group compared with those of untreated and CML28-transfected group. These results revealed the responder cells stimulated by SOCS1-silenced DCs underwent more times of division, indicating downregulation of SOCS1 expression remarkably enhanced stimulative capability of DCs and subsequent proliferation of response cell in MLR.

Transfected DCs induced CML28-specific CTL responses

We first determined whether DCs transfected with CML28 were capable of stimulating CML28-specific CTL responses against target cells *in vitro*. As demonstrated in Fig. 4a (right panel), CTLs induced by CML28-transfected DCs lysed both the CML28-transfected DCs and primary leukemia cells with different efficacy, whereas the PBMCs and untreated DCs were not apparently lysed. The stimulated CTL exhibited lower lytic efficacy against CML28-transfected DCs compared to those against primary leukemia cells, which can be partly explained by the transfection-efficacy-restricted proportion of CML28-positive ones among CML28-transfected DCs [4]. These findings suggest that the CML28-transfected DCs specifically induce CTL responses.

To investigate the effect of the SOCS1-silencing on priming the CTL response, we used SOCS1-silenced and CML28-transfected DCs to prime the CTLs, respectively. Compared with the CTLs responses induced by CML28-transfected DCs (right panel of Fig. 4a), we found that the lytic activities of the SOCS1-silenced group against the CML28-positive targets were significantly higher at various E:T ratios (left panel of Fig. 4a). Conversely, no apparent cross-reactivity against the untreated DCs and

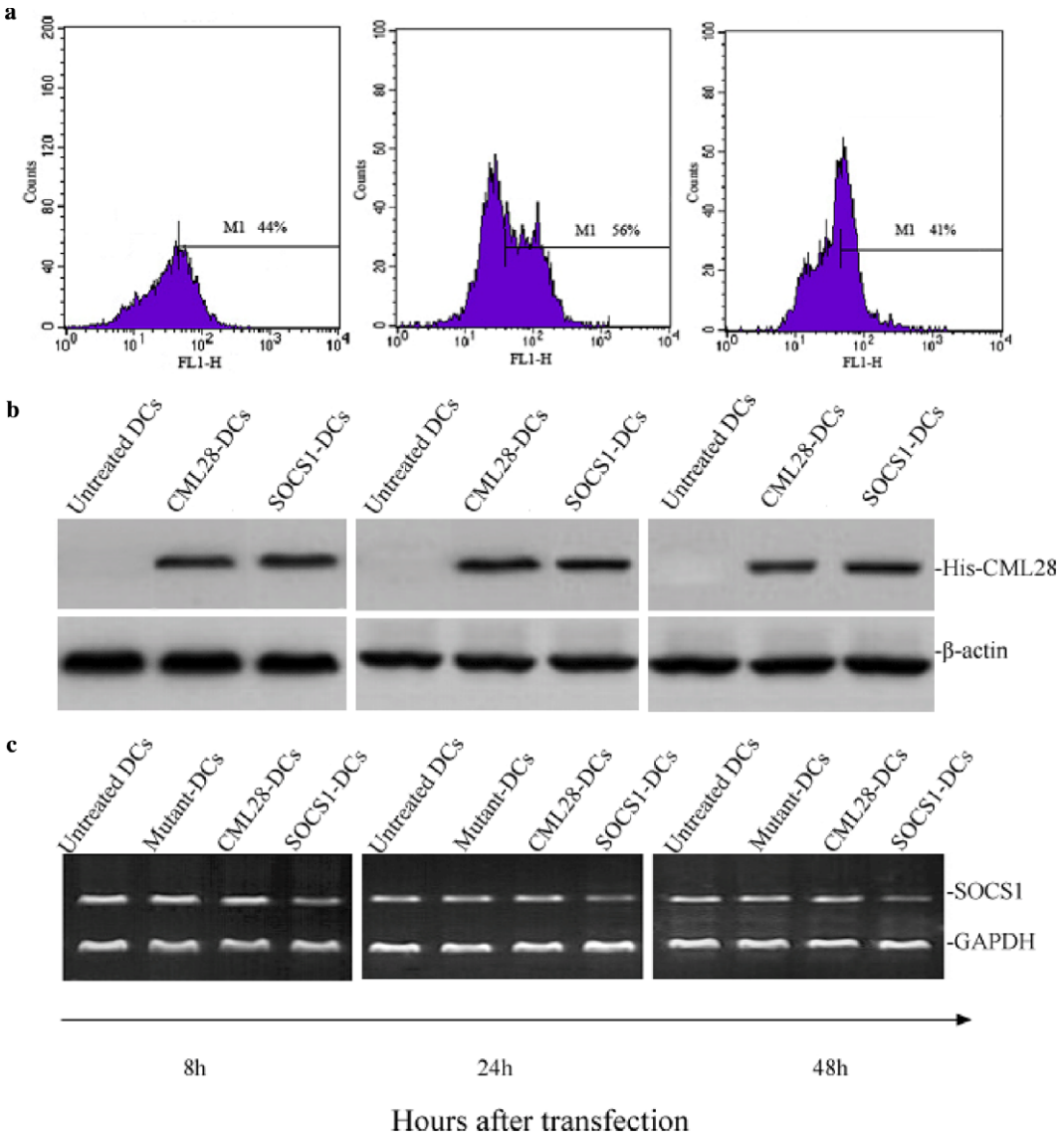

Fig. 1. Validation of expression of CML28 nucleic acid and efficiency of SOCS1 gene silencing. Transfected DCs were harvested at various time points (8, 24, and 48 h) after nucleofection and untreated DCs were used as control. Time course of EGFP expression in nucleofected DCs were measured by FACSCalibur analysis to evaluate the transfection efficiency (a). Number in the upper right indicates the percentage of EGFP-expressing cells. Western blot analysis of His-CML28 was performed as described in Materials and methods to verify the expression of His-CML28 fusion protein in transfected DCs (b). Semi-quantitative RT-PCR was performed as described and band intensities were measured and analyzed to evaluate the time-course of expression of SOCS1 mRNA post-siRNA treatment (c).

autologous PBMCs was detected (Fig. 4a). These results revealed that the SOCS1-silenced DCs were obviously superior to the SOCS1-expressing DCs in terms of initiating the CTL response.

Finally, to confirm the HLA restriction of target cell lysis, we used the HLA-mismatched cell lines, U937, HL60, and NK-sensitive K562 as target cells, which are all CML28-positive but HLA-I-mismatched (Table 1). In contrast with the HLA-matched target cells, we detected low level lysis against these target cells (Fig. 4b). The background levels of lytic activities exceed those of targeting untreated DCs and autologous PBMNCs, which might be attributed to limited nonspecific lysis of nature killer (NK) cell population due to HLA-I class mismatching. This result indicates that the CTL response induced by the transfected DCs was chiefly in a HLA-I-restricted manner.

Discussion

In this study, we demonstrated for the first time that DCs co-transfected with a CML28 nucleic acid-vaccination and SOCS1-specific siRNA expression vectors induce a CML28-specific CTL response. As a new TAA member, CML28 is likely to be a more promising target for anti-tumor immunotherapy with its broader spectrum expression in tumor cells and stricter expression in normal tissues, compared with other known TAAs. According to previous

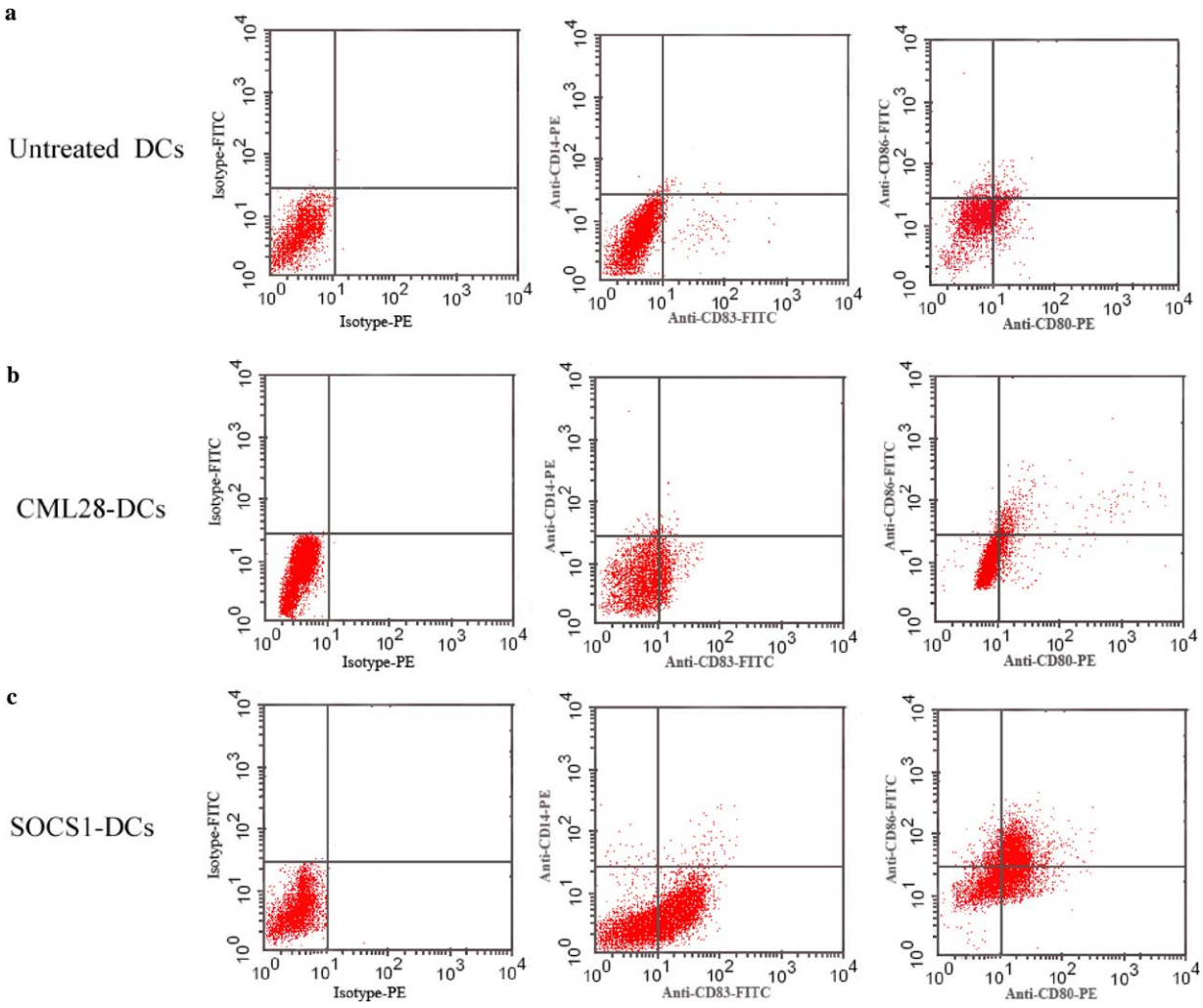

Fig. 2. SOCS1-silencing mediated by siRNA promoted maturation of DCs. Cultured cells at various time points were harvested for analyzing phenotypical change: the harvested immature DCs after seven days of culture with a cytokine cocktail (untreated DCs, a), CML28-transfected DCs (CML28-DCs, b) and SOCS1-silenced DCs (SOCS1-DCs, c) at 48 h post-electroporation. Cells were counterstained with monoclonal antibodies specific for CD14, CD83, CD80, and CD86. Nonreactive isotype-matched antibodies were utilized as controls (left panel).

study, CML28 is expressed at high levels in various kinds of primary leukemia cells including AML, CML-AP, and CML-BP, and at low levels in CML-CP cells [9]. Thus, effective induction of CML28-specific CTL responses in this manner provides a promising immunotherapy, potentially for a very broad population, including leukemia patients as well as other patients enduring more solid tumors.

Our data provide evidence that CTL induction by co-transfected DCs is feasible and effective in eliciting CML28-specific anti-tumor cytotoxicity. For defined TAAs, investigators have shown that cDNA-transfected DCs are as effective in their ability to induce antigen specific CTLs in vitro as DCs loaded with mRNA or antigen-derived peptides [11,21–23]. Given that the unfractionated tumor RNA-transfected DCs express the whole antigen repertoire of the tumor cell, the induced CTLs may represent a polyclonal population with potentially different antigen specificities and therefore the potential risk of autoimmunity [11]. The TAA-specific cDNA-transfected DCs only encode specific TAA proteins and not normal tissue antigens, so this strategy of loading TAA does not risk autoimmunity in theory. As presented herein, the CTLs were capable of lysing CML28-transfected DCs and primary leukemia cells, without any apparent lysis cross-reactivity against cognate PBMCs and untreated DCs. This indicates that autoimmunity was not induced in our study. Additionally, we confirmed the HLA-I-restricted manner of induced CTL response by HLA restriction assay.

We hereby verify and strengthen the recently proposed view that downregulation of SOCS1 in DCs greatly promotes DC maturation and T cell proliferative response, resulting in stronger lytic activities of subsequent CTL responses [6,7,24]. There is increasing evidence that DC is a double-edged sword in innate and adaptive immunity, inducing antigen-specific tolerance in the steady state or initiating specific T cell immunity against pathogens and tumors in activated state [1]. SOCS1 plays an important role in maintaining the tolerogenic state of DCs and negatively regulating DC maturation and antigen presentation

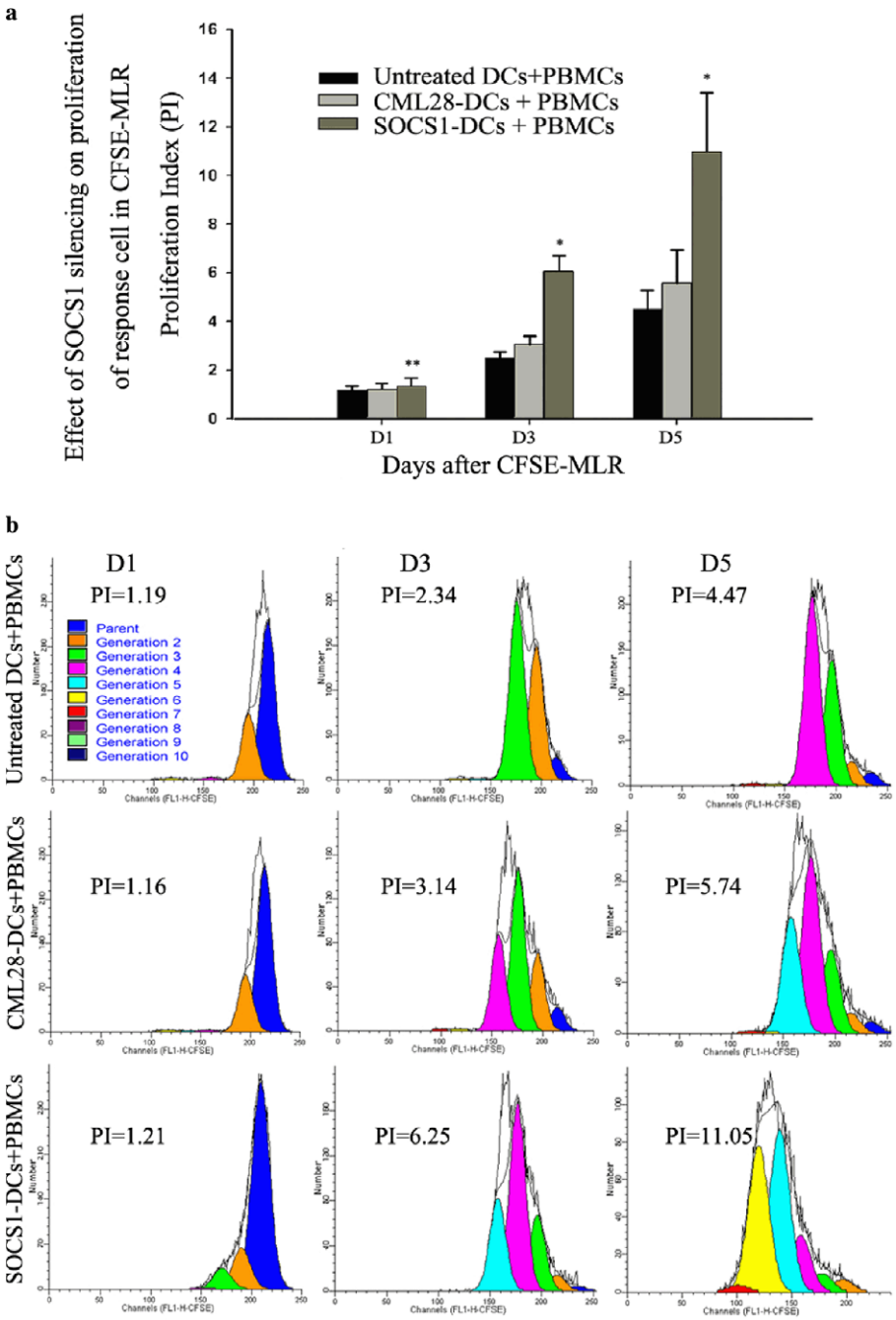

Fig. 3. Kinetics of the responder cell proliferation in CFSE proliferation assay. The nonadherent fraction of the PBMCs was labeled with CFSE as described and cocultured with untreated DCs, CML28-transfected DCs (CML28-DCs), and SOCS1-silenced DCs (SOCS1-DCs) in MLR culture, respectively. The FACS analysis of CFSE proliferation assay was performed on days one, three, and five. The CFSE-MLR data were analyzed on a proliferation wizard basic model program in MODfit (b) and were visualized with histograms to show the levels of proliferation index (a). $^{**}P > 0.05$, versus CML28-DCs/untreated DCs. $^{*}P < 0.01$, versus CML28-DCs/untreated DCs.

[5–7]. To ensure both the efficiencies of expression of CML28 nucleic acid and downregulation of SOCS1 by siRNA in DCs, we employ a new technique nucleofector™ to transfect hard-to-transfect DCs yielding 40–56% of transfection efficacy. SOCS1-silencing mediated by siRNA in our study might release the brake of SOCS1 on DCs maturation and activation, resulting in higher capability of antigen presentation and stimulatory effects of DCs, and leading to the following stronger CML28-specific lytic activities of CTLs. The rationale for using immature rather than mature DCs for CTLs priming is based on the evidence that immature DCs are a prerequisite for successful antigen uptake and processing [1]. Moreover, promoting maturation of DCs by SOCS1-silencing will enhance the stimulatory capacity of DCs and the subsequent magnitude of adaptive immunity initiated by DCs [5–7]. This

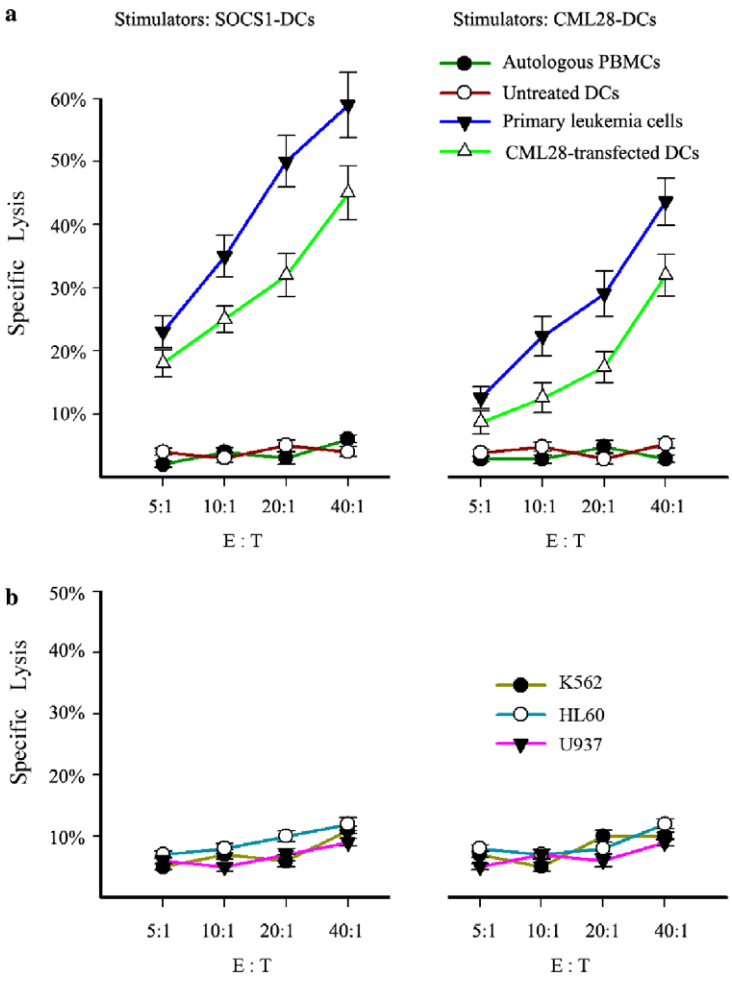

Fig. 4. Enhanced CML28-specific lysis induced by SOCS1-silenced DCs. DCs were generated from adherent PBMNCs of a healthy donor (Table 1) and then electroporated with CML28 nucleic acid vaccination and SOCS1 siRNA vector. The CTL induction was performed as described by CML28-transfected DCs (CML28-DCs, right panel) and SOCS1-silenced DCs (SOCS1-DCs, left panel), respectively. The cytolytic activity of the cells was determined in a standard ^{51}Cr-release assay, with the use of primary leukemia cell (Table 1), CML28-transfected DCs as targets, cognate PBMCs, and untreated DCs were used as control targets (a). $P < 0.05$, at the E:T ratio of 5:1, CML28-DC versus SOCS1-DC; $P < 0.01$, at the rest E:T ratios, CML28-DC versus SOCS1-DC. To confirm the HLA restriction lysis, U937, HL60, and K562 (Table 1) were used as control target cells (b).

Table 1
Characteristics of HLA class I alleles and CML28 expression of DC, CTL, and various targets

Cell/cell line	HLA-A	HLA-B	HLA-Cw	CML28
[a]DCs/CTLs	A02/24	B48/56	Cw12/14	–
[a]Primary leukemia cells	A02/24	B48/56	Cw12/14	+
U937	A11/—	B60/35	Cw04/07	+
HL60	A11/30	B13/58	Cw07/—	+
K562	A11/68	B60/18	NA[b]	+

[a] DCs/CTLs and primary leukemia cells were generated from HLA-matched sibling pair: donor and patient, respectively.
[b] NA, not available.

approach takes advantage of the functional distinction between immature and mature DCs and thus may provide a general and potent method for DC-based vaccination.

In conclusion, we show here for the first time the induction of CML28-specific CTL response by DCs co-transfected with the CML28 DNA vaccine and the SOCS1-specific siRNA expression vector. The strategy of immature DCs to initiate transfection and then promote maturation of DCs by SOCS1 silencing significantly contributes to our understanding of the CTL response. Given the broad spectrum expression of CML28, induction of CML28-specific CTL response will provide a promising immunotherapy for leukemia patients as well as solid tumor patients. Additional researches are needed to elucidate the detailed function of the CML28 gene as a novel TAA and newly identified component of the human exosome so-called proteasome for RNA [25–27].

Acknowledgments

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