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*J Immunol* 2010; 184:6084-6091; Prepublished online 26 April 2010; doi: 10.4049/jimmunol.0902561

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Transcription Factor E2F1 Suppresses Dendritic Cell Maturation

Fang Fang,*1† Yan Wang,*1† Rui Li,*† Ying Zhao,*† Yang Guo,*† Ming Jiang,*† Jie Sun,*† Yang Ma,*† Zijia Ren,*† Zhigang Tian,*† Feng Wei, † De Yang,†,‡ and Weihua Xiao*†‡

Transcription factor E2F1 has been largely studied as a promoter of S-phase transition in the cell cycle and as a regulator of apoptosis. Recently, E2F1 has been shown to regulate a wide range of genes in response to inflammatory stimulation of macrophages and to contribute to T cell activation in response to pathogens, implicating an extensive immunological role for E2F1. Dendritic cells (DCs) play critical roles as professional APCs in the development of immune responses. However, it is unclear whether E2F1 has any effect on DC phenotype or function. In this paper, we report that E2F1 acts as a suppressor of DC maturation. The level of E2F1 expression was transiently downregulated in the course of LPS-induced maturation of both human monocyte-derived DCs and a mouse DC cell line, DC2.4. Knockdown of E2F1 by small interfering RNA in DC2.4 cells resulted in both phenotypic and functional maturation, even without LPS treatment. Conversely, ectopic overexpression of E2F1 suppressed LPS-induced maturation of DC2.4 cells. Furthermore, knockdown of E2F1 caused the activation of several major signaling pathways known to be activated in the course of DC maturation, including Erk1/2, NF-kB, and PI3K/Akt, suggesting that E2F1 may be involved in regulating multiple signaling pathways in DCs. Finally, the alteration of phenotypic maturation by E2F1 was confirmed with bone marrow-derived DCs from E2F1 knockout mice. Overall, our data demonstrate for the first time that E2F1 is a critical regulator of DC maturation. The Journal of Immunology, 2010, 184: 6084–6091.

Dendritic cells (DCs) are the most potent professional APCs that control immunity (1). Under steady state, immature DCs populate peripheral tissues where they continuously sample the environment by endocytosis. Upon encountering pathogens and/or proinflammatory mediators, DCs undergo a complex transformation process termed “maturation,” which greatly enhances their Ag-presenting capacity. The general features of DC maturation involve the upregulation of surface costimulatory (e.g., CD80 and CD86) and MHC (class I and class II) molecules, the activation of lysosomal Ag-processing mechanisms, the production of numerous immunostimulatory cytokines, and the acquisition of the capacity to migrate to secondary lymphoid organs (1–3). Upon arriving at the secondary lymphoid organs, mature DCs present antigenic peptides displayed on their surfaces in the context of MHC molecules to naive T cells for the initiation of Ag-specific T cell immune responses, which is a unique capacity that only mature DCs possess. Therefore, controlling the maturation of DCs is critical for the regulation of adaptive immune responses.

DC maturation can be triggered by either exogenous agents, such as pathogen-associated molecular patterns (e.g., microbial DNA, RNA, LPS, etc.), or endogenous mediators, such as proinflammatory cytokines (e.g., TNF, IL-1, and IFN), CD40L, and alarmins. Most pathogen-associated molecular patterns and alarmins induce DC maturation by triggering certain pattern-recognition receptors, such as TLRs (1, 4–7). Proinflammatory cytokines stimulate DC maturation by signaling through their specific receptors, whereas CD40L does so by interacting with CD40, a member of the TNFR superfamily on the DC surface (1, 2, 7–10). Despite utilization of a variety of receptors, DC maturation in response to various ligands seems to stem from the activation of a number of intracellular signaling pathways including NF-κB, PI3K, and multiple MAPKs, which are collectively responsible for inducing the phenotypic and functional characteristics typical of mature DCs (2, 3, 7–9, 11, 12). NF-κB is the most frequently documented transcriptional factor, which plays critical roles in DC maturation (7, 10–13). Silencing RelB in DCs using small interfering RNA (siRNA) has been found to not only inhibit DC maturation but also downregulate DC-dependent development of Ag-specific immune responses (13). Alymphoplasia (Aly) mouse has a loss-of-function point mutant in the NF-κB–inducing kinase (14), and consequently, all cells in Aly mice, including DCs, are deficient in the activation of NF-κB via the

The Journal of Immunology

Received for publication August 7, 2009. Accepted for publication March 19, 2010.

This work was supported by grants from the National Natural Science Foundation of China (30721002 and 30528020), National Basic Research Program of China (973 Program) (2007CB914503), Ministry of Science and Technology of China (KSCX1-YW-R-58 and KSCX2-YW-R-174), and China Ministry of Education (20060358019). Funding to pay the open access publication charges for this article was provided by a grant from the National Basic Research Program of China (2007CB914503; to W.X.). This research was supported in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research, Frederick, Frederick, MD 21701; and Basic Science Program, Science Applications International Corporation-Frederick, National Cancer Institute at Frederick, Frederick, MD 21702.

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Abbreviations used in this paper: Aly, alymphoplasia; ASK1, apoptosis signal-regulating kinase 1; DC, dendritic cell; KO, knockout; MDC, macrophage-derived chemokine; pDNA, plasmid DNA; siRNA, small interfering RNA; SLC, secondary lymphoid-tissue chemokine; [3H]Tdr, [3H]thymidine deoxyribose; WT, wild-type.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0902561
noncanonical pathway (15). DCs of Aly mice show some deficiency in maturation and are unable to present exogenous Ags to naïve CD8 T cells (15). Only sporadic reports are available with respect to the participation of other transcriptional factors in the course of DC maturation, such as CREB, activating transcription factor-2, and AP-1 (10, 11).

Transcription factor E2F1 plays divergent roles in regulating gene transcription, the cell cycle, and apoptosis (16). E2F1 induces cell proliferation by advancing cells from the G0 phase to the S phase in the cell cycle (16–18). However, E2F1 can also induce apoptosis of different cell types through both p53-dependent and -independent pathways (16, 19, 20). E2F1−/− mice exhibit increased lymphoproliferation and the development of various tumors including lymphomas, suggesting that E2F1 has a proliferation-limiting role in lymphocytes by acting as a tumor suppressor (21, 22). A more recent study using E2F1 and E2F2 double-knockout (KO) mice not only demonstrated the profound effects of E2F1 and E2F2 on the proliferation and differentiation of lymphocytes and hematopoietic progenitor cells but also revealed an increased sensitivity of T cells to antigenic stimulation as well as signs of autoimmunity in these mice (23). Because Ag-specific lymphocyte proliferation in autoimmunity is likely initiated by mature, Ag-presenting DCs, the autoimmune activation of lymphocytes in E2F1 and E2F2 double-KO mice might be a result of the effects of E2F1 and E2F2 on DC development, differentiation, and/or maturation. Interestingly, the activation of macrophages induced by LPS, a TLR4 agonist often used for the stimulation of DC maturation, is accompanied by the recruitment of E2F1 to the promoters of many NF-kB–responsive genes (24). TNF, also capable of inducing DC maturation, has been shown to inhibit endothelial cell proliferation by downregulating E2F1 in a manner dependent on JNK and Rb phosphorylation (25). In addition, E2F1, through transcriptional induction of Gab2 and apoptosis signal-regulating kinase 1 (ASK1), respectively, regulates the activation of PI3K/Akt and Erk1/2 signaling pathways (26, 27). PI3K/Akt and Erk1/2, two key signal transduction pathways (16, 19, 20). E2F1−/− mice display a strongly enhanced maturation phenotype compared with the control mice. Thus, our data demonstrate a potent suppressive role for E2F1 and E2F2 in maturation and are unable to present exogenous Ags to naïve CD8 T cells (15). Only sporadic reports are available with respect to the participation of other transcriptional factors in the course of DC maturation, such as CREB, activating transcription factor-2, and AP-1 (10, 11).

Flow cytometry

The expression levels of surface marker molecules were measured by immunostaining and flow cytometry using a FACS caliber instrument (BD Biosciences, San Jose, CA). FACs data were analyzed using Win MDI 2.9 software. For immunostaining, 1 × 10^6 cells were stained with FITC- or PE-conjugated Abs or isotype controls. The following PE-conjugated anti-mouse mAbs were used: anti-CD11c and anti-CD83 (eBioscence, San Diego, CA). The following FITC-conjugated anti-mouse mAbs were used: anti-HLA-DR (BD Biosciences) and anti-CD86 (eBioscence). All flow cytometric analyses were performed using appropriate isotype controls, and the data were collected for 10,000 cells/sample. The percentages of positive cells were analyzed using a two-way factorial ANOVA in which LPS treatment and cell types (E2F1 siRNA and control) were the factors.

Migration assay

For in vitro studies, DC2.4 (10^5) in serum-free medium was placed in a Transwell migration chamber (Costar 3421; Corning Glass, Corning, NY) and allowed to migrate through a polycarbonate membrane (pore size, 5 μm) at 37°C. Secondary lymphoid-tissue chemokine (SLC; 100 ng/ml)
(PeproTech) was placed in the lower chamber to induce cell chemotaxis. After 6 h, the wells were removed, and the cells on the top of the membrane were wiped off with a cotton swab. Cells on the bottom of the membrane were fixed with 3.7% formaldehyde for 15 min, stained with 0.5% Triton X-100 for 3 min, and stained with 1% Hoehchest at 37°C for 6 min. The cells were counted using a light microscope. Images of cell migration under various conditions were photographed. The numeric data represented the means of three independent tests (each in triplicates) and SE (2±SE). For in vivo studies, DC2.4/Scramble and DC2.4/E2F1-KD cells were injected into the left and right hind footpads of C57BL/6 mice, respectively. After 36 h, popliteal lymph nodes were removed to make single-cell suspensions for the analysis of CSFE-positive cells by flow cytometry.

Measurement of cytokine and chemokines
Supernatants of cultured DCs were harvested and kept at −80°C. The concentrations of selected cytokines and chemokines were measured using the multiplex protein array (Thermo Scientific, Rockford, IL) as described previously (6).

T cell proliferation
Splenocytes were prepared from the spleens of 8-wk-old BALB/C mice. Stimulator cells were grown on a 12-well plate in the presence or absence of LPS (1 µg/ml) for 48 h. The cells were harvested, washed twice, suspended at 10^7/ml, and irradiated at 8000 rad. The stimulator cells were diluted with the prepared splenocytes in a ratio of 1:5 to 1:3125 and cocultured for 4 d. [3H]Thymidine (1 µCi) was then added into each culture well, and the culture wells were allowed to culture overnight. At the end, the cells were harvested, and a liquid scintillation counter was used to measure the incorporation of [3H]thymidine. The data represent three tests with triplicate samples in each test.

Generation, treatment, and analysis of bone marrow-derived DCs in mice
Bone marrow cells were flushed out from the tibias and femurs of 6- to 8-wk-old female E2F1 wild-type (WT) or KO mice (purchased from The Jackson Laboratory, Bar Harbor, ME). Bone marrow progenitors were isolated and cultured in RPMI 1640 medium containing 20 ng/ml mouse GM-CSF (PeproTech) at 10^7/ml for 6 to 8 d to generate DCs as described previously (6). DCs obtained on day 8 were analyzed for the expression of costimulatory and MHC class II molecules by flow cytometry. To compare the maturational responses of WT and E2F1-KO DCs, DCs obtained on day 6 were cultured in the presence of LPS (200 ng/ml) for 22 h and subsequently analyzed by flow cytometry after immunostaining.

Results
E2F1 expression is transiently suppressed during LPS-induced DC maturation
As the first step in examining the role of E2F1 in DC maturation, we determined whether the expression of E2F1 was altered in the course of maturation of human monocyte-derived DCs upon treatment with LPS. DCs treated with 100 ng/ml LPS for 24 h showed a much higher expression of surface CD86, MHC class II, and CD83 (a critical marker of DC maturation) than DCs cultured in the absence of LPS, verifying that the concentration of LPS used was adequate in inducing DC maturation (Fig. 1A). When monitored by RT-PCR, DCs in the course of LPS-induced maturation exhibited a time-dependent change in E2F1 mRNA expression (Fig. 1B). E2F1 expression began to decrease as early as 3 h, gradually reaching its lowest level at 12 h, and then started to recover at 18 h to 24 h after LPS addition (Fig. 1B). The transient downregulation of E2F1 mRNA in the course of LPS-induced maturation of monocyte-derived DCs was also confirmed by quantitative PCR (Fig. 1C).

To facilitate investigation of the role of E2F1 in DC maturation, we used DC2.4, an immature DC cell line established previously from the culture of C57BL/6 bone marrow progenitors, as a model system (30). As shown in Fig. 2A, surface expression of mouse MHC class II, CD86, CD83, and CD11c after treatment with 1 µg/ml LPS for 24 h was upregulated in DC2.4 cells as demonstrated by overlying histograms of flow cytometric analysis, indicating that LPS treatment was capable of inducing the maturation of DC2.4 cells. Subsequently, the kinetics of E2F1 expression in DC2.4 cells in response to LPS-induced maturation was examined by RT-PCR (Fig. 2B), real-time PCR (Fig. 2C), and Western blot analysis (Fig. 2D). Upon LPS treatment, E2F1 expression at the mRNA level in DC2.4 cells exhibited a kinetic change similar to that of LPS-induced maturation of human monocyte-derived DCs: a transient downregulation beginning at 3 h, reaching its lowest level at 6 to 12 h and then the start of recovery at 18 h after LPS addition (Fig. 2B, 2C). Western blot analysis confirmed that upon LPS-induced maturation, E2F1 expression at the protein level in DC2.4 cells showed a similar transient downregulation, albeit with a lag of 2 to 3 h (Fig. 2D). Therefore, the expression of E2F1 was transiently suppressed during LPS-induced DC maturation in both human and mouse DCs, suggesting a potentially important role of E2F1 in DC maturation.

Knockdown of E2F1 enhances phenotypic maturation of DCs
To further investigate the role of E2F1 in DC maturation, we generated a DC2.4 cell line with its endogenous E2F1 knocked down (designated hereafter as DC2.4/E2F1-KD) and compared its phenotype with control cells (designated as DC2.4/Scramble). In comparison with DC2.4/Scramble cells, DC2.4/E2F1-KD cells exhibited a much lower E2F1 expression at both mRNA (Fig. 3A) and protein levels (Fig. 3B), indicating that the knockdown was effective. Interestingly, DC2.4/E2F1-KD cells showed remarkably higher expressions of surface MHC class II, CD86, CD83, and CD11c than DC2.4/Scramble cells, suggesting that reducing the level of E2F1 might promote maturation of DCs (Fig. 3C). The upregulation of various surface markers on DC2.4/E2F1-KD is unlikely to be the result of the transfection per se, because DC2.4/Scramble cells had similar levels of surface costimulatory and MHC class II expression as parental DC2.4 cells (data not shown).
DC maturation is characterized by three phenotypic hallmarks: 1) upregulation of surface costimulatory (e.g., CD83 and CD86) and MHC molecules; 2) increase in their capacity to produce proinflammatory mediators (e.g., cytokines and chemokines); and 3) acquisition of the ability to respond to lymphoid tissue homing chemokine, such as ELC/CCL19 or SLC/CCL21 (1–3). To determine whether knockdown of E2F1 results in full maturation of DC2.4 cells or just upregulation of cell surface costimulatory and MHC molecules, we investigated the capacity of DC2.4/E2F1-KD cells to produce proinflammatory mediators and to migrate in response to SLC/CCL21. After DC2.4/Scramble and DC2.4/E2F1-KD cells were induced to mature with LPS and their production of selected cytokines and chemokines in the culture supernatants was measured by protein array, DC2.4/E2F1-KD cells were found to produce higher levels of IL-12, TNF-α, Rantes, and macrophage-derived chemokine (MDC) than the scramble control cells in response to LPS stimulation (Fig. 3D). The kinetics of IL-10 and IL-6 production were either similar or slightly delayed for E2F1 knockdown cells (Fig. 3D). Of note, the basal level of IL-12 was significantly elevated in E2F1 knockdown DC2.4 cells in comparison with the control cells, but this was not the case with other tested cytokines. These data suggest that knockdown of E2F1 enhances the capacity of DC2.4 cells to produce proinflammatory cytokines (e.g., IL-12 and TNF-α) and chemokines (e.g., Rantes and MDC) upon LPS-induced DC maturation.

To examine the effect of E2F1 knockdown on DC migratory/homing capacity, DC2.4/E2F1-KD and DC2.4/Scramble cells were investigated both in vitro and in vivo for their migration in response to SLC/CCL21. E2F1 knockdown in DC2.4 cells enhanced the expression of surface marker molecules and production of cytokines. DC2.4 cells were transfected with a vector expressing either siRNA specific for E2F1 or a scramble sequence (as control) and selected under G418 to establish two stable cell lines, DC2.4/E2F1-KD and DC2.4/Scramble. The E2F1 expression by these two cell lines was measured by RT-PCR at the mRNA level (A) and Western blot analysis at the protein level (B). The surface expression of selected marker molecules was determined by flow cytometry (C). The production of selected proinflammatory cytokines and chemokines in the supernatants was measured by multiplex protein array after stimulating the cells with LPS at 100 ng/ml for the time period specified (D).
to CCL21/SLC and for their homing ability to draining lymph nodes, respectively. A comparison of the migration of DC2.4/E2F1-KD and DC2.4/Scramble cells in response to SLC/CCL21 revealed that SLC, at the concentration used, induced the migration of DC2.4/E2F1-KD cells but not DC2.4/Scramble cells (Fig. 4A, B). In addition, analysis of accumulated CSFE-labeled DC2.4/E2F1-KD and DC2.4/Scramble cells injected into the left and right hind footpads of mice, respectively, in the popliteal lymph nodes revealed that many DC2.4/E2F1-KD, but not DC2.4/Scramble, cells reached draining lymph nodes 36 h after injection (Fig. 4C). These results indicate that E2F1 knockdown led to DC2.4’s acquisition of the capacity to migrate toward SLC in vitro and to home in on draining lymph nodes in vivo.

**Knockdown of E2F1 enhances functional maturation of DCs**

Maturation of DCs is accompanied by the acquisition of enhanced capacity to present Ag(s) and migration (1, 2). To determine the effect of E2F1 knockdown in DC2.4 cells on their Ag-presenting capacity, we compared the capacity of DC2.4/E2F1-KD cells with DC2.4/Scramble cells to stimulate the proliferation of T cells in an allogeneic MLR assay. Splenocytes from BALB/c mice were used as responder T cells because of the C57BL/6 origin of DC2.4 cells. DC2.4/Scramble cells had a very low stimulatory capacity (Fig. 5, ♦). However, DC2.4/Scramble treated with LPS, as expected, developed the capacity to stimulate the proliferation of allogeneic T cells (Fig. 5, ●). DC2.4/E2F1-KD cells (Fig. 5, △) exhibited a remarkable enhanced capacity to stimulate the proliferation of allogeneic T cells as compared with DC2.4/Scramble cells (Fig. 5, ♦), indicating that knockdown of E2F1 greatly enhanced the Ag-presenting capacity of DC2.4. Of note, LPS treatment of DC2.4/E2F1-KD cells (Fig. 5, △) did not enhance their capacity to stimulate the proliferation of allogeneic T cells (Fig. 5, compare △ with ●), suggesting that knockdown of E2F1 caused full maturation of DC2.4 at the functional level. Therefore, knockdown of E2F1 resulted in both phenotypic and functional maturation of DC2.4 cells.

**Overexpression of E2F1 suppresses DC maturation**

To further demonstrate the suppressive effect of E2F1 on DC maturation, we also determined the effect of E2F1 overexpression on DC phenotype and function. DC2.4 cells were transfected with vector (pcDNA) alone or with pcDNA-E2F1, followed by selection with G418 to establish DC2.4/pcDNA and DC2.4/pcDNA-E2F1 cell lines. Overexpressions of E2F1 by DC2.4/pcDNA-E2F1 cells were confirmed by RT-PCR (Fig. 6A) and Western blot analysis (Fig. 6B). When analyzed for the expression of surface marker molecules by flow cytometry, DC2.4/pcDNA-E2F1 cells expressed much less MHC class II, CD86, and CD83 than DC2.4/pcDNA cells (Fig. 6C), indicating that excessive E2F1 inhibited the maturation of DC2.4 cells. In addition, DC2.4/pcDNA and DC2.4/pcDNA-E2F1 cells, either untreated or treated with LPS to stimulate maturation, were tested in allogeneic MLR to determine the effect of overexpression of E2F1 on the functional maturation of DCs (Fig. 6D). DC2.4/pcDNA cells matured by LPS treatment stimulated robust proliferation of allogeneic splenocytes at a DC:splenocyte ratio higher than 1:125, suggesting that they could be activated into functionally mature DCs upon LPS treatment (Fig. 6D). In contrast, DC2.4/pcDNA-E2F1 cells failed to stimulate proliferation at all DC:splenocyte ratios tested.

**FIGURE 4.** Effect of E2F1 knockdown on the migratory capacity of DC2.4 cells. The in vitro migration of DC2.4/Scramble and DC2.4/E2F1-KD cells in response to SLC (100 ng/ml) was determined by chemotaxis assay. Shown are the representative images (original magnification ×200) of cell migration under different conditions from one representative experiment (A) and the average (mean ± 2*SE) number of cell migrations from three separate experiments (B). To determine in vivo homing, CSFE-labeled DC2.4/Scramble and DC2.4/E2F1-KD cells were injected into the left and right hind footpads of C57BL/6 mice, respectively. After 36 h, popliteal lymph nodes were removed to analyze the level of CSFE-positive cells by flow cytometry (C).

**FIGURE 5.** E2F1 knockdown resulted in the functional maturation of DC2.4 cells. DC2.4/Scramble and DC2.4/E2F1-KD cells were incubated in the absence or presence of LPS (1 µg/ml) for 48 h before they were harvested and gamma irradiated. Subsequently, various numbers of BALB/c splenocytes in the indicated ratio with irradiated DC2.4 cells (5 × 10^5) were mixed and added into a 96-well, flat-bottomed, tissue culture plate (in triplicate). The plate was incubated at 37°C in humidified air containing 5% CO_2 for 96 h, with the addition of [^3]H[TdR (0.5 µCi/well) for the last 18 h. The cells were harvested and the incorporation of [^3]H[TdR was measured by beta scintillation counting. Shown is the average cpm (means ± SD) of triplicate wells of one experiment representative of three. [^3]H[TdR, [^3]H thymidine deoxyribose.
pcDNA-E2F1 cells, even after LPS treatment, did not stimulate the proliferation of allogeneic mouse lymphocytes at any DC:splenocyte ratios tested, indicating that overexpression of E2F1 suppressed LPS-induced functional maturation of DC2.4 cells (Fig. 6D). Thus, data from both knockdown and overexpression experiments supported the notion that E2F1 appears to be a critical suppressor of DC maturation.

Alteration of E2F1 in DC2.4 cells influences multiple signaling pathways

Maturation of DC involves a complicated cellular transition process that is associated with the activation of multiple signaling pathways. To gain some insight into how E2F1 suppresses DC maturation, several major signaling pathways that are considered critical for DC maturation were examined. The cell lysate from two sets of stably transfected DC2.4 cells with either knockdown (Fig. 7A) or overexpression (Fig. 7B) of E2F1 together with their corresponding controls were subjected to Western blot analysis. As shown in Fig. 6, the activation of several intracellular signal transducers (as indicated by the level of phosphorylated proteins) including Erk1/2, Akt, and NF-κB p65 were markedly enhanced by E2F1 knockdown as compared with control cells (Fig. 7A). Conversely, their levels of activation in DC2.4 cells were reduced upon E2F1 overexpression (Fig. 7B). In contrast, the level of phospho-p38 was decreased in E2F1 knockdown cells but increased in E2F1 overexpression cells, in comparison with corresponding levels in the control cells. E2F1 knockdown or overexpression did not appear to affect the expression of unphosphorylated ERKs, p38, Akt, and NF-κB p65, as evidenced by similar bands between E2F1 knockdown or overexpressed

FIGURE 7. Effect of E2F1 knockdown or overexpression on the activation of multiple signal transducers in DC2.4 cells. Western blot analysis of the total and phosphorylated forms of selected intracellular signal transducers (including ERKs p38, Akt, and NF-κBp65) were performed with cell lysate from two sets of DC2.4 cells with either E2F1-knockdown (A) or E2F1 overexpression (B) and their corresponding control cells that were established and used in Fig. 6. Lane 1, DC2.4/Scramble; lane 2, DC2.4/E2F1-KD; lane 3, DC2.4/pcDNA; and lane 4, DC2.4/pcDNA-E2F1. GAPDH was used as a loading control.

FIGURE 8. Comparative analysis of the maturational status of DCs generated from E2F1 WT and KO mice bone marrow progenitors. DCs were differentiated from bone marrow mononuclear cells by culturing in the presence of 20 ng/ml mouse GM-CSF for 6 to ∼8 d. A. BM-derived DCs on day 8 (from both WT and KO) were immunostained with FITC- or PE-conjugated Abs against mouse CD83, CD80, CD86, or MHC class II (closed area) or isotype-matched control Abs (open area) and analyzed by flow cytometry. B. Bone marrow-derived DCs on day 6 were treated with 200 ng/ml LPS for 22 h before they were stained and analyzed as in A. The numbers inside the histograms are the geometric mean of relative fluorescence of corresponding surface molecules. Shown are the overlay histograms of one experiment representative of two.
DC2.4’s with their corresponding control cells (Fig. 7). Thus, E2F1 regulates the activation of many signal transduction pathways that are associated with DC maturation.

**E2F1 KO DCs show a more mature phenotype**

To determine the physiologic relevance of our findings, we generated DCs from the bone marrow of E2F1 KO and WT control mice and measured the expression of a number of surface molecules. E2F1 KO DCs cultured in vitro for 8 d exhibited higher expression of CD86, CD80, MHC class II, and CD83 than E2F1 WT DCs generated under identical condition (Fig. 8A). Moreover, when day 6 DCs were treated with LPS for 22 h, E2F1 KO DCs upregulated higher levels of all surface molecules measured than identically treated E2F1 WT DCs (Fig. 8B). These data are consistent with the results of E2F1 knockdown or overexpression experiments and collectively demonstrate the suppressive effect of E2F1 on DC maturation.

**Discussion**

The term “DC maturation,” originally coined to define the acquisition of Ag-presenting activity by murine epidermal Langerhans cells after isolation from skin (33), is used to describe the overall phenotypic and functional transformations of DCs triggered by diverse exogenous and endogenous mediators, such as TLR ligands (e.g., LPS), proinflammatory cytokines, alarmins, and CD40L (1–5). Despite advances in studies on DC maturation, the identity of the transcription factors that play critical roles in the course of DC maturation is still not fully understood. Previous studies have shown that several transcription factors including NF-κB, AP-1, activating transcription factor-2, and CREB, are involved in the course of DC maturation (7, 9, 10, 12, 13, 15). Our present study demonstrates that E2F1 is another transcription factor that controls DC maturation. First, in the course of LPS-induced maturation of human monocyte-derived DCs, as well as mouse DC2.4 DCs, the level of E2F1 underwent a transient downregulation in maturing DCs both at the mRNA and protein levels (Figs. 1, 2). In addition, knockdown of E2F1 expression in DC2.4 cells modified their phenotypic and functional features to those of mature DCs, including upregulation of surface costimulatory and MHC molecules, enhanced production of various proinflammatory cytokines (e.g., IL-12p70 and TNF-α) and chemokines (e.g., Rantes and MDC), acquisition of the capacity to respond to lymphoid-homing chemokine SLC to allow them to home in on draining lymph nodes, and augmented Ag-presenting capacity (Figs. 3–5). Furthermore, overexpression of E2F1 in DC2.4 cells decreased their surface expression of maturation markers and LPS-induced enhancement of Ag-presenting capacity (Fig. 6). Although unsuccessful attempts were made to do knockdown with primary DCs due mainly to low transfection rates, nevertheless, E2F1 KO DCs showed a more mature phenotype than E2F1 WT DCs, demonstrating the physiologic relevance of our findings (Fig. 8). Therefore, E2F1 acts as a suppressor of DC maturation and, to our knowledge, is the first transcription factor to be identified as a negative regulator of DC maturation.

The maturation of DCs in response to numerous stimuli is accompanied by the activation of several intracellular signaling transducers including NF-κB, PI3K, and members of the MAPK superfamily (2, 6–9, 11, 12). In support of the role of E2F1 as a suppressor of DC maturation, knockdown of E2F1 in DC2.4 cells resulted in the activation of ERKs Akt (indicative of PI3K activation) and NF-κB, whereas overexpression of E2F1 in DC2.4 cells resulted in a reduction in their activation (Fig. 7). Because p38 MAPK is also known to be activated during DC maturation, it was therefore expected that knockdown of E2F1 (by inducing DC maturation) would enhance, whereas E2F1 overexpression would decrease, the activation of p38 MAPK in DC2.4 cells. Surprisingly, its activation in DC2.4 cells was reduced by E2F1 knockdown and enhanced by E2F1 overexpression (Fig. 7). Although it is unclear why this seemingly paradoxical phenomenon occurred in DC2.4 cells, it might be due to the direct promoting effect of E2F1 on the activation of p38 MAPK. In this regard, several previous studies using DNA screening microarrays have shown that E2F1 upregulates the level of ASK1, a member of the MAPK kinase family capable of activating the p38 MAPK pathway in diverse cell types (34–36). Recently, E2F1 has been shown to promote the activation of p38 MAPK through induction of ASK1 in both osteosarcoma cells and human lung fibroblasts (27). It remains to be determined whether E2F1 indeed regulates the activity of ASK1 in DC2.4 cells.

The E2F1 transcription factor plays diverse roles in cells ranging from promoting cell proliferation in certain cell types to promoting cell death by apoptosis in other cell types (16–20, 22, 31). The identification of E2F1 as a suppressor of DC maturation in the current study adds another role to the panoply of functions ascribed to E2F1. The participation of E2F1 in DC maturation is suggested by a very recent paper reporting that E2F1 and several other transcription factors including NF-κB, AP-1, AP-2, SP1, and early growth response 1 are involved in the activation of monocytes and DCs induced by alarmin LL-37 (37). The identification of E2F1 as a suppressor of DC maturation suggests that E2F1 may act as an anti-inflammatory or immunosuppressive transcription factor. In this regard, E2F1 has previously been shown to suppress TNF-α-induced activation of human aortic endothelial cells by inhibiting NF-κB activation and translocation (38). Furthermore, mice lacking E2F1 are reported to be more susceptible to the development of autoimmune syndromes (23, 39, 40). In these mice, there are excessive numbers of mature CD4 and CD8 T cells and reduced number of CD4+CD25+ regulatory T cells, which is thought to be due to the result of a defect in the apoptosis of T cells (23, 40). However, with the identification of E2F1 as a suppressor of DC maturation in the current study, we can speculate that lack of E2F1 could augment maturation of DCs carrying self-Ags and enhance the activation of autoreactive lymphocytes, reducing the generation of inducible regulatory T cells in E2F1 KO mice.

**Acknowledgments**

We thank Dr. Joost J. Oppenheim (Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, National Cancer Institute at Frederick, MD) for helpful discussion and critical reading of the manuscript.

**Disclosures**

The authors have no financial conflicts of interest.

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