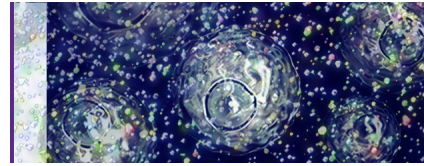


Focus on a Single Target or Measure Up to 13 at Once

With BioLegend Immunoassays

Learn More



Activation of Dendritic Cells via Inhibition of Jak2/STAT3 Signaling

Yulia Nefedova, Pingyan Cheng, Daniele Gilkes, Michelle Blaskovich, Amer A. Beg, Said M. Sebti and Dmitry I. Gabrilovich

This information is current as
of March 5, 2021.

J Immunol 2005; 175:4338-4346; ;
doi: 10.4049/jimmunol.175.7.4338
<http://www.jimmunol.org/content/175/7/4338>

References This article **cites 24 articles**, 7 of which you can access for free at:
<http://www.jimmunol.org/content/175/7/4338.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>



Activation of Dendritic Cells via Inhibition of Jak2/STAT3 Signaling¹

Yulia Nefedova,* Pingyan Cheng,* Daniele Gilkes,* Michelle Blaskovich,* Amer A. Beg,[†] Said M. Sebti,* and Dmitry I. Gabrilovich^{2*}

Signaling via Jak2/STAT3 is critically important for normal dendritic cell (DC) differentiation. In addition, we have previously demonstrated that hyperactivation of the Jak2/STAT3 pathway induced by tumor-derived factors (TDF) may be responsible for abnormal DC differentiation in cancer. In this study, using a novel selective inhibitor of Jak2/STAT3, JSI-124, we investigated the mechanism of the Jak2/STAT3 effect on DCs and the possibility of pharmacological regulation of DC differentiation in cancer. Our experiments have demonstrated that JSI-124 overcomes the differentiation block induced by TDF and promotes the differentiation of mature DCs and macrophages. Surprisingly, inhibition of Jak2/STAT3 signaling resulted in dramatic activation of immature DCs generated in the presence of TDF as well as in control medium. This activation manifested in up-regulation of MHC class II, costimulatory molecules, and a dramatic increase in the ability to stimulate allogeneic or Ag-specific T cells. Inhibition of Jak2/STAT3 signaling resulted in activation of the transcription factor NF- κ B. This up-regulation was not due to a conventional pathway involving I κ B α , but was probably due to a block of the dominant negative effect of STAT3. This indicates that Jak2/STAT3 play an important role in negative regulation of DC activation, and pharmacological inhibition of the Jak2/STAT3 pathway can be used to enhance DC function. *The Journal of Immunology*, 2005, 175: 4338–4346.

Dendritic cells (DCs)³ are specialized APCs that recognize, acquire, process, and present Ags to naive resting T cells for the induction of an Ag-specific immune response (1–3). DCs are critically important for the induction and maintenance of antitumor immune responses, both spontaneously developed and induced as a result of immunotherapy. Inadequate function of the host immune system may render all attempts to use immunotherapy ineffective. Data from many different laboratories obtained during the past few years indicate that a defect in the DC system is one of the main factors responsible for tumor escape (reviewed in Ref. 4). Recent studies have demonstrated an important role of the Jak2/STAT3 pathway in DC differentiation under physiological conditions and in cancer. Laouar et al. (5) reported that STAT3 is required for Flt3 ligand-dependent DC differentiation. At the same time, we have demonstrated that hyperactivation of Jak2/STAT3 signaling is directly involved in the abnormal DC differentiation in cancer (6, 7). Myeloid cells maintaining high levels of Jak2 and STAT3 activity were not able to differentiate into DCs in vitro (7).

Jaks and STAT proteins are critical components of diverse signal transduction pathways that are actively involved in cellular survival, proliferation, differentiation, and apoptosis (8). Jaks are constitutively associated with many cytokine and growth factor receptors, including those implicated in defective DC differentiation (reviewed in 9). Activated Jaks eventually induce phosphorylation of STATs, followed by their translocation into the nucleus, where they modulate the expression of target genes. We hypothesized that inhibition of tumor-induced Jak2/STAT3 hyperactivation in myeloid cells may improve DC differentiation and function and, ultimately, the antitumor immune response.

To test this hypothesis we used a new selective inhibitor of the Jak2/STAT3 pathway, JSI-124 (cucurbitacin I). We have previously demonstrated that JSI-124 selectively inhibited the activation of Jak2 and STAT3, but not Src, Akt, ERK, or JNK (10). JSI-124 inhibited the growth of tumors with constitutively active STAT3, but did not affect tumors without STAT3 hyperactivation (10).

This study, for the first time, demonstrates that inhibition of Jak2/STAT3 signaling dramatically improves differentiation of DC. Surprisingly, inhibition of Jak2/STAT3 resulted in dramatic activation of DCs. This effect was observed in control DCs as well in cells generated in the presence of tumor-derived factors (TDF). It appears that the main mechanism of the effect of STAT3 activation inhibitors on DC activation was up-regulation of NF- κ B, not through a conventional mechanism of phosphorylation and degradation of I κ B, but, rather, through elimination of the dominant negative effect of STAT3 on NF- κ B.

Materials and Methods

Reagents, drug, and cell culture

RPMI 1640, DMEM, FBS, and antibiotics were obtained from Invitrogen Life Technologies, recombinant murine GM-CSF and IL-4 were purchased from RDI, LPS and Con A were obtained from Sigma-Aldrich, and Low-Tox rabbit complement and Lympholyte M were purchased from Cedarlane Laboratories. Lysine-fixable FITC-dextran (FITC-DX; m.w., 40,000) was obtained from Molecular Probes and dissolved in PBS. All peptides were purchased from SynPep. The following Abs were obtained from BD

*Immunology and Drug Discovery Programs, H. Lee Moffitt Cancer Center, and the Department of Interdisciplinary Oncology, University of South Florida, Tampa, FL 33647; and [†]Department of Biosciences, Columbia University, New York, NY 10027

Received for publication March 18, 2005. Accepted for publication July 26, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by National Institutes of Health Grants CA84488 and CA100062 (to D.G.) and 5P01CA78038 (to S.M.S.) and in part by the Flow Cytometry Core and Molecular Imaging Core at H. Lee Moffitt Cancer Center. Y.N. was supported by National Institutes of Health Fellowship F32CA103393.

² Address correspondence and reprint requests to Dr. Dmitry I. Gabrilovich, H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL 33612. E-mail address: dgabril@moffitt.usf.edu

³ Abbreviations used in this paper: DC, dendritic cell; BM, bone marrow; CM, conditioned medium; FITC-DX, FITC-dextran; HA, hemagglutinin; HPC, hemopoietic progenitor cell; IMC, immature myeloid cell; LN, lymph node; TDF, tumor-derived factor; VC, vehicle control (DMSO); TBS/T, TBS/Tween20.

Pharming: anti-Gr-1 (anti-Ly-6G), anti-CD11b, anti-CD11c, anti-I-A^b, anti-I-A^d, anti-CD86, anti-CD40, anti-I-A/I-E, and anti-CD3. Anti-F4/80 Ab were purchased from Serotec. JSI-124 (cucurbitacin I) was obtained from National Cancer Institute. For *in vivo* experiments, cucurbitacin I was obtained from Indofine Chemicals. It was dissolved in DMSO.

Murine NIH-3T3 fibroblasts and the CT26 colon carcinoma cell line were obtained from American Type Culture Collection. Cells were grown in DMEM supplemented with 10% FBS and antibiotics. The methylcholantrene-induced sarcoma cell line was obtained from Dr. L. J. Old, Ludwig Institute for Cancer Research, New York. Methylcholantrene-induced tumor was developed in BALB/c mice and passaged *in vivo* as an ascetic tumor. To generate conditioned medium (CM), cells were kept in medium with a reduced (3%) FBS concentration. After 48 h, supernatants were collected, filtered, and used in experiments.

Generation of DCs and isolation of cells

Bone marrow (BM) cells were obtained from the femurs and tibias of mice, and red cells were eliminated using ACK buffer. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 20 ng/ml GM-CSF, 10 ng/ml IL-4, and 50 μ M 2-ME, alone or in the presence of control (from 3T3 fibroblasts) or tumor cell (from CT26) CM. Half of the medium was replaced every 2 days. Cells were collected on day 5, followed by isolation of CD11c-positive DCs. To investigate the effect of JSI-124 on hemopoietic progenitor cell (HPC) differentiation, BM cells obtained from mice were initially enriched for HPCs by depletion of lineage-specific cells using an Ab mixture against T cells (TIB 210 and TIB-207), B cells (B220), macrophages and DCs (TIB-120), granulocytes (Gr-1), and red cells (Ter-119) as described previously (11). HPCs were cultured for 5–7 days as described above for BM culture. Gr-1- or CD11c-positive cells were isolated from spleens of tumor-bearing or control mice using a magnetic bead separation technique and MidiMACS according to the manufacturer's protocol (Miltenyi Biotec). The purity of Gr-1- or CD11c-positive populations was >95%, as determined by flow cytometry.

Evaluation of T cell proliferation and cytokine production

Murine CD11c DCs were used as stimulators in allogeneic MLR. T cells isolated from spleens of allogeneic mice using T cell enrichment columns (R&D Systems) were used as responders. Cells were mixed at different ratios and incubated in triplicate in U-bottom, 96-well plates for 4 days. [³H]Thymidine (1 μ Ci/well; Amersham Biosciences) was added 18 h before cell harvest. [³H]Thymidine incorporation was measured using a liquid scintillation counter. In some experiments splenocytes were cultured for 4 days in the presence of 1 or 5 μ g/ml Con A or 0.5 μ g/ml anti-CD3 Ab. The Ag-specific T cell response was evaluated using MHC class II (I-A^d)-restricted (SFERFEIPKE) influenza virus hemagglutinin (HA)-derived peptide. Murine DCs were cultured with autologous T cells isolated from spleens of TCR transgenic mice in the presence of 12.5 μ g/ml HA-derived peptide. In some experiments, after 2 days of incubation, cells were collected, and intracellular levels of IFN- γ , IL-10, and IL-2 were determined in the population of CD4⁺ T cells by flow cytometry using the manufacturer's protocol (BD Pharmingen). Cells were analyzed on a FACSCalibur using CellQuest software (BD Biosciences).

To evaluate whether the direct contact or soluble factors were responsible for induction of the T cell response, DCs were cultured in U-bottom, 96-well plates mixed with splenocytes or on the top of 0.2- μ m Transwell inserts (Nunc). After 4 days in culture, cells were pulsed with BrdU for 1 h, collected, labeled with anti-CD3 Ab, fixed, and permeabilized using a BrdU Flow Kit (BD Pharmingen), followed by staining with anti-BrdU-FITC Ab and 7-aminoactinomycin. The proportion of CD3⁺ T cells in the S phase of the cell cycle was evaluated by flow cytometry.

FITC-DX assay

The FITC-DX assay was performed as described previously (12). Cells were collected and resuspended in RPMI 1640 medium buffered with 25 mM HEPES and supplemented with 10% FBS. FITC-DX was added at a final concentration of 1 mg/ml, and cells were then incubated at 37°C or on ice. After 45 min, cells were washed with ice-cold PBS containing 1% FBS and analyzed on a FACSCalibur (BD Biosciences). For each sample the background (mean value of fluorescence of cells pulsed at 4°C) was subtracted from the mean value of fluorescence of cells incubated at 37°C.

ELISA

CD11c⁺ DCs were plated in a concentration of 10⁶ cells/ml and treated with JSI-124 or DMSO (vehicle control (VC)) for 48 h. After that time, supernatants were collected, and the levels of IL-12 (p70) and RANTES

were measured using ELISA kits (Pierce Endogen and BioSource, respectively). The sensitivity of both assays was >5 pg/ml.

ELISPOT assay

An ELISPOT assay was performed as described previously (13). Briefly, MultiScreen-HA plates (Millipore) were precoated with anti-mouse IFN- γ Ab (BD Pharmingen) by overnight incubation at 4°C. Two hundred thousand splenocytes were plated in quadruplicate in each well and cultured for 24 h at 37°C in the presence of MHC class I-matched (H2K^d) control p53-derived (KYICNSSCM) or specific HA-derived (IYSTVASSL) peptides (10 μ g/ml). Cells were then washed with PBS containing 0.1% Tween 20, and plates were incubated overnight at 4°C with biotinylated anti-IFN- γ Ab (BD Pharmingen). Results were visualized using avidin-alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate (Sigma-Aldrich). The number of spots was calculated on a CTL analyzer (CTL Analyzers) using ImmunoSpot 2.8 version software (Cellular Technology). Results are presented as the number of spots per 1×10^6 cells.

EMSA and Western blotting

EMSA was performed as described previously (11). Specific bands were visualized by overnight exposure to x-ray films (Fuji) at -70°C. Quantitation of band intensity was performed on Storm phosphorimager using ImageQuant software (Molecular Dynamics). For Western blotting, cells were lysed in radioimmunoprotection assay buffer. An equal amount of protein was run on an SDS-PAGE gel, followed by transfer onto a nitrocellulose membrane. The membrane was blocked in 5% milk in TBS/Tween20 (TBS/T) for 1 h at room temperature and probed overnight at 4°C with the following primary Abs: anti-p65, c-Rel, RelB, I κ B α , or β -actin (Santa Cruz Biotechnology), phospho-STAT3 or STAT3 (Cell Signaling Technology), or anti-mouse IA/IE-biotin Ab (BD Pharmingen). Membranes were then washed with TBS/T, incubated with corresponding secondary Abs (all obtained from Santa Cruz Biotechnology, excluding anti-biotin-HRP Ab, which was purchased from Cell Signaling), and then washed again with TBS/T. Specific bands were visualized using the chemiluminescence reagent kit (ECL; Amersham Biosciences), and their intensities were quantitated using ImageQuant software.

Immunoprecipitation

Cells were collected, washed twice with ice-cold PBS, and lysed in the following buffer: 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 2 mM Na₃VO₄, 2 mM PMSF, and 1/100 protease inhibitor mixture (Sigma-Aldrich). STAT3 Ab was added to lysates (500 μ g of protein/sample) and incubated with end-over-end rotation for 2 h at 4°C, followed by the addition of protein A-Sepharose beads (Amersham Biosciences) and incubation for another 1 h under the same conditions. Protein/Ab/beads mix was then washed five times with lysis buffer, resuspended in Laemmli SDS sample buffer, and denatured at 95°C for 5 min. Samples were resolved in 8% SDS-PAGE, followed by transfer to a polyvinylidene difluoride membrane. After blocking, the membrane was probed with p65, RelB, c-Rel, or STAT3 Abs. The membrane was developed using the ECL detection kit.

Luciferase assay

A luciferase assay was performed to evaluate NF- κ B transcriptional activity in DCs as previously described (11). Briefly, 5×10^6 CD11c⁺ DCs were electroporated with either the 6 \times IFN- γ tLuc plasmid containing the luciferase reporter gene under a NF- κ B-dependent promoter or the control pGL₃-basic plasmid containing only the luciferase gene. Cotransfection with pRL-thymidine kinase plasmid containing *Renilla* luciferase gene was performed to measure the transfection efficiency. After that, cells were treated with JSI-124 or VC for 36 h, followed by a luciferase assay using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity values for specific and control plasmid were normalized to *Renilla* luciferase activity.

PCR

Total cellular RNA was extracted using the RNeasy Mini kit (Qiagen). First-strand cDNA was synthesized with 1 μ g of RNA using SuperScript II reverse transcriptase (Invitrogen Life Technologies) and oligo(dT) primers according to the manufacturer's protocol. PCR was performed using CHITA promoter I- and promoter III-specific primers. Primers were gifts from Dr. K. Wright (Moffitt Cancer Center, Tampa, FL). Amplification of the *hprt* gene served as an internal control. Each PCR carried appropriate positive and negative controls. The products were electrophoresed on 1.5% agarose gel and photographed.

Confocal microscopy

Cells were collected on slides using cytospin centrifugation, fixed in 4% paraformaldehyde in PBS for 10 min, and washed three times with PBS, followed by permeabilization in 0.5% Triton X-100 for 5 min and blocking for 30 min in 2% BSA in PBS. After that, slides were incubated in a humid chamber for 1 h with primary biotinylated anti-IA/IE Ab, followed by incubation with streptavidin-FITC Ab. Slides were analyzed on the Zeiss LSM 510 confocal microscope.

Statistics

Statistical analysis was performed using JMP software (SAS Institute).

Results

Inhibition of the Jak-2/STAT3 pathway improves differentiation of DCs from HPCs

Previous studies have determined that JSI-124 inhibits STAT3 activation in tumor cells (10). In this study we investigated whether this compound exerts similar effects on STAT3 activation in DCs. HPCs isolated from control mice were grown in the presence of control (3T3) or tumor cell (CT26) CM for 5 days. CD11c⁺ DCs were isolated and treated with 0.5 μ M JSI-124 or VC for 24 h. This dose was selected after preliminary experiments. It significantly inhibited STAT3 activity (the level of pSTAT3; Fig. 1A) and provided >95% viability of DCs (data not shown). Consistent with our previous observations (7), STAT3 activity in DCs generated in the presence of TDF was substantially higher than that generated in the presence of control CM. JSI-124 significantly reduced the level of phospho-STAT3 in both groups of cells (Fig. 1B).

Accumulation of immature myeloid cells (IMC) and their inability to differentiate into DCs is one of the hallmarks of immu-

nological abnormalities in tumor-bearing hosts. We asked whether inhibition of STAT3 signaling might affect the differentiation of these cells. Enriched HPCs isolated from BM of control mice were cultured for 7 days with GM-CSF and IL-4. Medium alone or CM from CT26 tumor cells or control 3T3 fibroblasts was added on day 0, and JSI-124 (0.5 μ M) or VC was added on day 3. As expected, the presence of TDF significantly increased the proportion of Gr-1⁺CD11b⁺ IMC and decreased the proportion of CD11c⁺IA^{d+} and CD11c⁺CD86⁺ DCs (Fig. 1C). Treatment with JSI-124 did not affect the cell populations generated in the presence of medium alone or 3T3 CM. However, it significantly decreased the proportion of IMCs and increased the proportion of DCs generated in the presence of CT26 tumor cell CM. These data were consistent with our previous observations made using the dominant negative STAT3 construct (7) and suggested that inhibition of Jak2/STAT3 signaling allowed IMCs to differentiate toward DCs. However, greater differences were observed when we evaluated the level of expression of MHC class II and CD86 within the gated population of CD11c⁺ cells. Treatment with JSI-124 dramatically increased the expression of these molecules (Fig. 1D). This suggested that JSI-124 might not only promote DC differentiation, but also activate DCs.

Inhibition of Jak2/STAT3 signaling activates DCs

To directly evaluate this possibility, DCs were obtained by isolation of CD11c⁺ cells after 5-day culture of HPC with GM-CSF and IL-4 in the presence of 3T3 or CT26 CM. These DCs were then cultured with medium alone, 3T3, or CT26 CM for an additional 4 days in the presence of JSI-124 or VC. JSI-124 induced

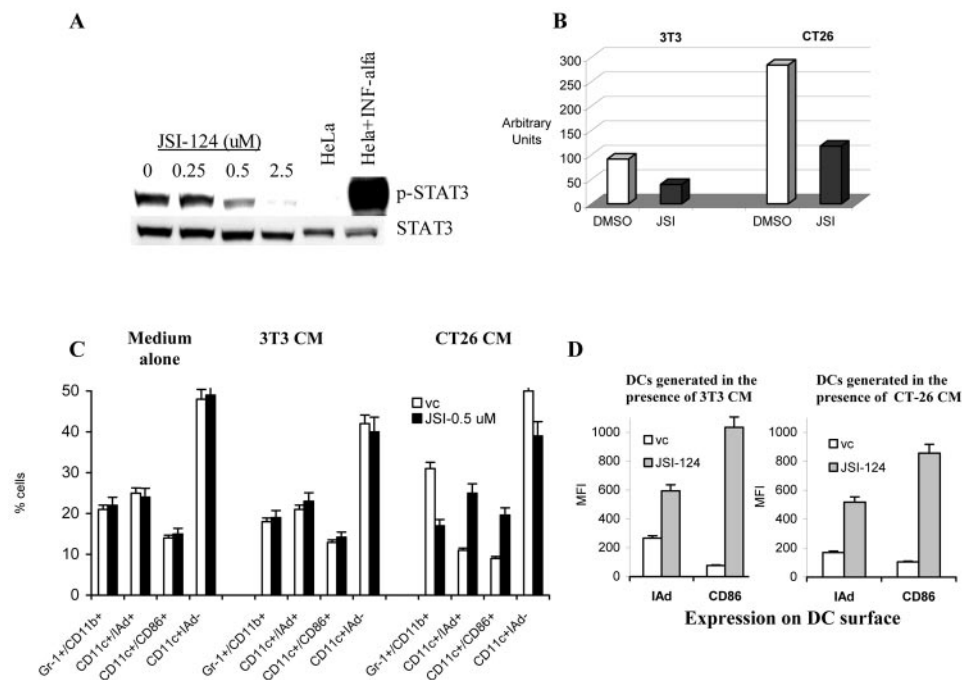


FIGURE 1. JSI-124 decreases the levels of phospho-STAT3 and promotes DC differentiation. *A*, DC were generated from HPCs in the presence of tumor cell (CT26) CM for 5 days. CD11c⁺ DCs were isolated and treated with different concentrations of JSI-124 for 24 h. Proteins were evaluated by Western blotting. HeLa cells treated with IFN- α were used as a positive control for STAT3 activation. *B*, HPCs were grown in the presence of control (3T3) or tumor cell (CT26) CM for 5 days. CD11c⁺ DCs were isolated and treated with 0.5 μ M JSI-124 or VC for 24 h. The intensities of specific bands were quantitated using ImageQuant software. The intensities of the signals from pSTAT3 were normalized to that from total STAT3. No differences in the level of total STAT3 between the cells were observed. *C*, HPCs were isolated from mouse BM and cultured in medium alone or in the presence of 3T3 or CT26 CM. Cells were treated with JSI-124 or VC starting from day 3. On day 7, cells were collected. The proportions of the various cell populations were calculated. The mean results of four experiments are shown. *D*, CD11c⁺ cells from the experiment described in *C* were gated, and the level of expression of MHC class II (IA^d) and B7-2 (CD86) molecules was evaluated. The mean results of four experiments are shown.

substantial (2- to 4-fold) up-regulation of the expression of MHC class II, B7-2 (CD86), and CD40 molecules on the surface of DCs (Fig. 2, *A* and *B*). The expression of surface markers and the effect of JSI-124 were the same in cells cultured in medium alone and those cultured in 3T3 CM (data not shown). JSI-124 dramatically up-regulated the ability of DCs to stimulate allogeneic T cells (Fig. 2*C*) or Ag-specific T cells (Fig. 2*D*). Inhibition of the Jak2/STAT3 pathway in DCs resulted in loss of the ability to take up soluble Ag. DCs generated in the presence of CT26 CM had a significantly higher level of FITC-DX uptake than those generated in the presence of 3T3 CM, which reflects their immature state. JSI-124 dramatically down-regulated FITC-DX uptake in both cases (Fig. 2*E*). Taken together, these observations indicate that inhibition of the Jak2/STAT3 pathway resulted in activation/maturation of DCs. We evaluated the ability of DCs to induce a peptide-specific im-

mune response *in vivo*. DCs generated from BM precursors were cultured for 4 days with either VC or JSI-124 pulsed with specific peptide and injected into BALB/c mice twice at a 10-day interval. Seven days after the second immunization, mice were killed, and the peptide-specific response was measured in an IFN- γ ELISPOT assay. Treatment of DCs with JSI-124 substantially increased their ability to induce a CD8⁺ T cell response (Fig. 2*F*).

The presence of endotoxin in the JSI-124 preparation could activate DCs and thus affect the interpretation of the results. JSI-124 is a pure natural product and not a bacterial product; therefore, its contamination by endotoxins is highly unlikely. We tested the level of endotoxin contamination directly. In a *Limulus* amoebocyte lysate test (CapeCode), an undiluted 0.5- μ M solution of JSI-124 showed endotoxin activity of <0.03 endotoxin units/ml (detection limit).

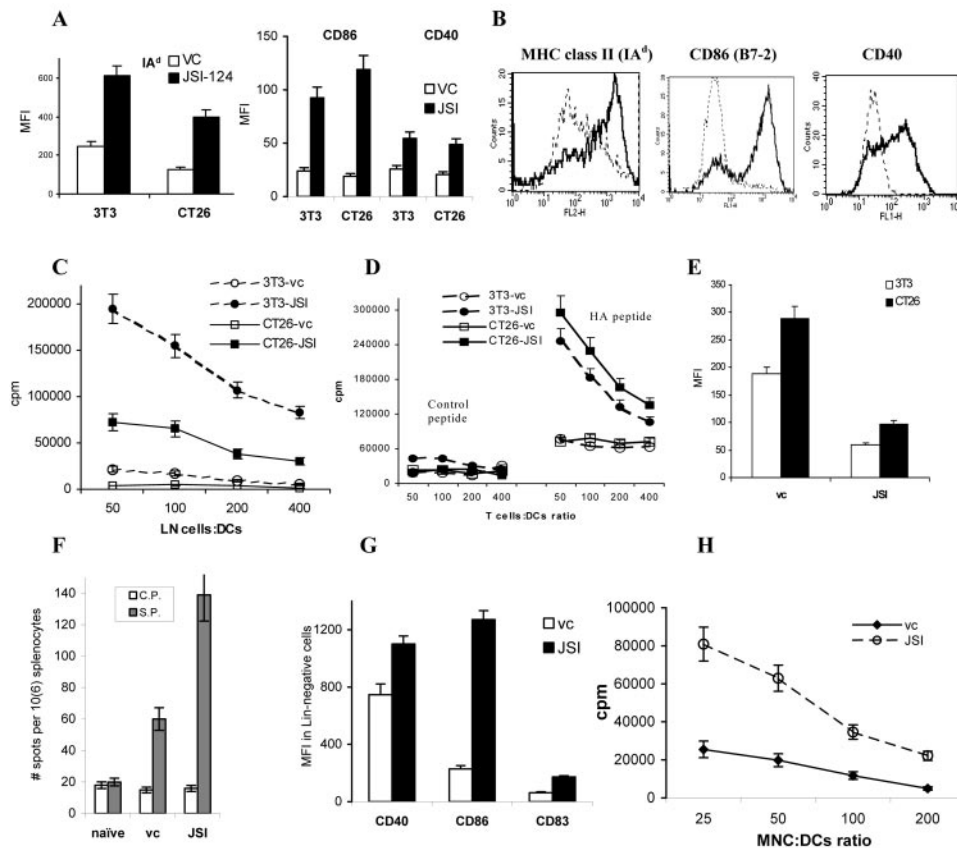


FIGURE 2. The Jak/STAT3 pathway inhibitor JSI-124 activates DCs. DCs were generated from mouse BM cells in the presence of control 3T3 or CT26 CM. CD11c⁺ DCs were isolated using the magnetic beads separation technique. Cells were then treated with 0.5 μ M JSI-124 or VC and cultured again with 3T3 or CT26 CM. On day 4, cells were collected, and their phenotype and function were evaluated. The level of surface marker expression was evaluated by flow cytometry. The mean results of three experiments performed (*A*) and a typical example (*B*) are shown. MFI, mean fluorescence intensity. The dotted line represents CD11c⁺ DCs cultured for 4 days with DMSO; the solid line represents CD11c⁺ DCs treated with JSI-124. The expressions of MHC class II (IA^d), CD86, and CD40 molecules were evaluated by flow cytometry as described in *Materials and Methods*. Cells were also used as stimulators of allogeneic T cells in MLR (*C*) and of syngeneic T cells in the presence of HA peptide (*D*). Two experiments with the same results were performed. *E*, The ability of DC to process Ag was evaluated by uptake of FITC-DX. The level of FITC-DX uptake at 4°C was subtracted from uptake at 37°C. The mean results of three experiments performed are shown. *F*, DCs were generated from BM HPC using GM-CSF and IL-4. CD11c⁺ cells were isolated and treated for 4 days with either 0.5 μ M JSI-124 or VC. After that time DCs were pulsed with 10 μ g/ml H2K^d matched HA-derived peptide. Naive BALB/c mice were immunized s.c. with 2×10^5 DCs. Immunization was repeated once, 10 days later. Seven days after the second immunization, mice were killed, and splenocytes were isolated and stimulated with either control (C.P.) or specific (S.P.) peptide. The number of IFN- γ -producing cells was measured in an ELISPOT assay. Each group included three mice, and each experiment was performed in quadruplicate. Naive, nonimmunized mice. The mean \pm SD are shown. *G* and *H*, Human DCs were generated *in vitro* from peripheral blood MNC with GM-CSF and IL-4 and purified by metrizamide gradient centrifugation. Cells were then treated with 0.5 μ M JSI-124 or VC for 5 days. *G*, Then DCs were collected and labeled with PE-conjugated lineage mixture Ab (anti-CD3, CD14, CD19, and CD56), PerCP-conjugated anti-HLA-DR Ab, and FITC-conjugated anti-CD86, CD40, and CD83 Abs. Cells were analyzed by flow cytometry (FACSCalibur). Fluorescence intensity was measured within the population of lin⁻ HLA-DR⁺ DCs. *H*, DCs treated with JSI-124 or VC were mixed in triplicate in U-bottom, 96-well plates at different ratios with 10^5 MNC from a different donor. Cells were incubated for 5 days. [³H]thymidine was added 18 h before cell harvest. The mean results of two experiments are shown. Spontaneous proliferation of MNC was <3000 cpm.

Next, we asked whether the inhibition of Jak2/STAT3 signaling had an effect on human DCs. Mononuclear cells were obtained from the peripheral blood of healthy volunteers, and DCs were generated using GM-CSF and IL-4 as previously described (14). DCs were treated with 0.5 μ M JSI-124 or DMSO for 5 days. Cells were collected, and their phenotype and function were analyzed. DCs were defined as lineage mixture-negative (CD3, CD14, CD19, and CD56) and HLA-DR-positive cells. JSI-124 induced a significant increase in the expression of CD40 and CD83 molecules on the DC surface, but an especially dramatic increase was observed in the expression of CD86 (Fig. 2G). DCs treated with JSI-124 had considerably greater ability to stimulate allogeneic T cells than control cells (Fig. 2H). These data indicate that JSI-124 exerts similar effects on DCs in humans as it does in mice.

Activated DCs exert their effect on T cells via direct cell-cell contact

Previous studies have demonstrated that inhibition of STAT3 in macrophages and tumor cells with dominant negative forms of STAT3 or conditional knockout results in increased production of different cytokines, most prominently RANTES (6, 15). In this study we measured the levels of RANTES and IL-12 (p70), the cytokine critically important for DC function. JSI-124 increased RANTES production by DCs >5-fold (Fig. 3A). However, inhibition of Jak2/STAT3 signaling in DC did not affect IL-12 production. It remained below detectable levels in all experiments performed (data not shown). We asked whether increased functional activity of JSI-124-treated DCs was due to soluble factors released by these cells. CD11c⁺ DCs generated from BM cells of BALB/c mice were treated with JSI-124 or DMSO for 4 days. Splenocytes from syngeneic BALB/c mice were placed in the bottom chamber of 96-well Transwell plates and stimulated with 0.5 μ g/ml anti-CD3 Ab. DCs were added either directly to the bottom chamber or in the top chamber. JSI-124-treated DC increased anti-CD3-induced T cell proliferation >6-fold (Fig. 3B). However, this effect was observed only when DCs were placed in direct contact with T cells, but not in the top chamber of the Transwell plates. To make sure that our data are accurate, we used a different assay (BrdU pulse labeling of responder cells) and a different experimental system with Ag-specific T cells. Lymph node (LN) cells isolated from HA-transgenic mice were placed in the bottom

chamber of 96-well plates. DCs were added either directly to the bottom chamber or to the upper chamber. LN cells were stimulated with HA-derived specific peptide, and T cell proliferation was evaluated 4 days later. JSI-124-treated DCs significantly increased the Ag-specific T cell response. This augmentation was observed only when DCs were added directly to the LN cells, but not when DCs were separated by semipermeable membrane (Fig. 3C). This indicates that direct cell-cell contact was required for JSI-124-treated DCs to exert their effect on T cell activation.

Mechanism of MHC class II up-regulation on DCs by Jak2/STAT3 inhibitor

As we demonstrated above, DC activation induced by JSI-124 manifested in a dramatic up-regulation of MHC class II on the cell surface. There are two potential mechanisms of this effect: inhibition of Jak2/STAT3 could result in increased synthesis of MHC class II molecules, or MHC class II molecules could be transferred from the cytoplasm to the cell surface. To address this question, CD11c⁺DCs were generated from BM cells and treated with 0.5 μ M JSI-124 or VC. Total RNA was extracted from cells cultured for 24 and 48 h, followed by RT-PCR with primers specific for MHC CIITA promoters I and III. Two-day treatment of DCs with JSI-124 completely blocked CIITA promoter I expression. As expected, CIITA promoter III expression was barely detected in myeloid DCs (Fig. 4A). These data were confirmed by the analysis of MHC class II expression in Western blotting with anti-I-A/I-E Abs (Fig. 4B) and indicated that not only did JSI-124 not stimulate MHC class II transcription and synthesis, but, in fact, it blocked MHC CIITA, which eventually results in shutting down MHC class II transcription. Experiments with intracellular staining of DCs with MHC class II Ab showed that JSI-124 induced translocation of MHC class II molecules from intracellular compartments to the cell surface (Fig. 4C).

The transcription factor NF- κ B is one of the major factors responsible for DC maturation. In our experiments, JSI-124 induced activation of NF- κ B, as measured by DNA binding in an EMSA assay (Fig. 5A) and transcriptional activity of the reporter gene (Fig. 5B). Importantly, strong activation became evident only 72 h after the start of the treatment (Fig. 5A). The main conventional

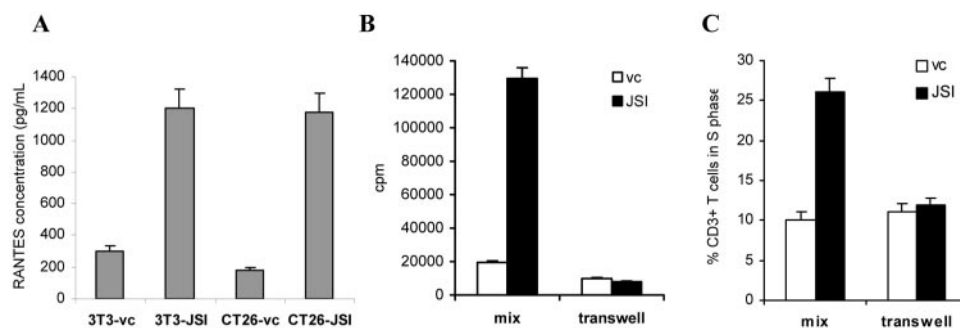


FIGURE 3. When activated by JSI-124, DCs exert their effect via direct cell-cell contact. *A*, The RANTES concentration was measured in triplicate in supernatants obtained after 48-h treatment of CD11c⁺ DC with JSI-124 or VC as described in *Materials and Methods*. *B*, Splenocytes were isolated from syngeneic BALB/c mice, and 2×10^5 cells were placed in the bottom chamber of U-bottom, 96-well Transwell plates. Cells were stimulated with 0.5 μ g/ml anti-CD3 Ab. DCs treated with VC or JSI-124 (JSI) were added to either the bottom chamber (2×10^3 cells/well; mix) or the upper chamber (4×10^3 cells/well; Transwell). [³H]Thymidine was added 18 h before cell harvest, and its incorporation was measured on a scintillation counter as described in *Materials and Methods*. All experiments were performed in triplicate and repeated at least once to assure reproducibility of the results. *C*, LN cells were isolated from HA-TCR transgenic mice and were placed into the bottom chamber of Transwell wells (10^5 cells/well). Cells were stimulated with 12.5 μ g/ml specific HA-derived peptide. DCs were placed into the bottom chamber (10^3 /well) or the upper chamber (2×10^3) of the Transwell. After 4-day culture, the upper chamber was removed, and cells were pulsed for 1 h with BrdU. The proportion of CD3⁺ T cells in the S phase of the cell cycle was evaluated by flow cytometry. Each experiment was performed in triplicate. Two experiments with the same results were performed.

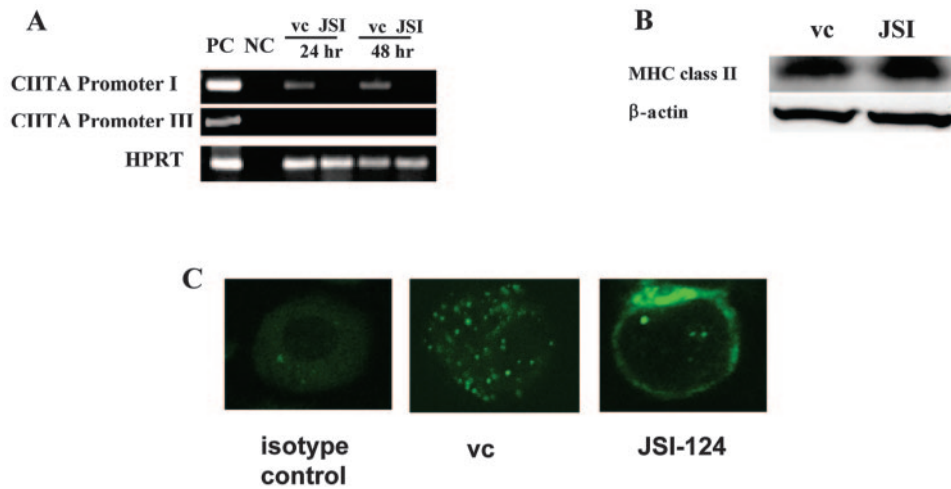


FIGURE 4. JSI-124 induces translocation of MHC class II to the surface of DCs. Murine BM HPCs were cultured with GM-CSF and IL-4 to generate DCs. After 5-day culture, CD11c⁺ cells were isolated using the magnetic beads separation technique and were then treated with 0.5 μ M JSI-124 or VC. **A**, Total RNA was isolated from cells cultured for 24 and 48 h, followed by RT-PCR as described in *Materials and Methods*. **B**, Cells were collected after 48 h in culture, and Western blotting was performed using I-A/I-E Ab as described in *Materials and Methods*. **C**, After 48 h in culture, cells were collected onto cytospin slides. Slides were fixed and stained with either isotype control IgG (control) or biotinylated anti-I-A/I-E Ab, followed by streptavidin-FITC, then analyzed by confocal microscopy ($\times 1000$).

pathway of NF- κ B activation is the phosphorylation and degradation of I κ B α . Fig. 5C illustrates the effect of LPS on I κ B α degradation in DCs. However, JSI-124 did not affect I κ B α (Fig. 5D). It also did not affect the expression of NF- κ B subunits (Fig. 5E). Thus, it appears that JSI-124 did not activate NF- κ B via I κ B α degradation or increased synthesis of NF- κ B subunits.

Mechanism of NF- κ B activation by Jak2/STAT3 inhibition

One of the possible mechanisms that may regulate NF- κ B activity was suggested recently. STAT3 was shown to bind p65 in the cytoplasm and thus prevent its translocation to nuclei and DNA binding (16). We tested the possibility of a physical interaction between STAT3 and NF- κ B subunits in DCs and the effect of JSI-124 on this process. DCs were generated in the presence of tumor-derived factors, as described above, and then were stimulated with a known activator of NF- κ B, TNF- α . STAT3 were pulled down, and membranes were probed with Abs against different members of the NF- κ B family. We could detect coprecipitation of STAT3 with c-Rel, but not with p65 (Fig. 6A) or RelB (data not shown). Next, DCs were treated with VC or JSI-124 instead of TNF- α . Treatment of DCs with JSI-124 completely eliminated the presence of c-Rel from the STAT3 complex (Fig. 6B). These experiments, although suggestive, demonstrated a rather low level of c-Rel protein coprecipitated with STAT3. To independently verify these data, we performed a supershift EMSA with Abs against p65 and c-Rel. Consistent with previous data, stimulation of DCs with TNF- α resulted in nuclear translocation of both p65 and c-Rel subunits of NF- κ B (Fig. 6C). These subunits were absent in unstimulated DCs treated with DMSO (Fig. 6D). However, treatment of DCs with JSI-124 induced a marked increase in DNA binding of c-Rel and very little in p65 (Fig. 6D). These data also suggest that STAT3 may interact with c-Rel in DCs, and therefore, inhibition of STAT3 phosphorylation by JSI-124 may prevent the formation of such complexes.

To test the functional significance of this hypothesis, we investigated the effect of JSI-124 on DCs isolated from c-Rel-deficient mice. These mice do not have a defect in DC production, and DCs generated from these mice have a normal response to LPS (17). In our experiments, CD11c⁺DCs were generated from BM HPCs and

then treated with JSI-124 or DMSO for 5 days. No significant differences in the level of expression of MHC class II, B7-2, or CD40 were observed in VC-treated DCs obtained from *c-Rel*^{-/-} or *c-Rel*^{+/+} mice. In *c-Rel*^{+/+} mice, JSI-124 induced a significant increase in the expression of MHC class II and CD86 and a slightly lower, but still significant, increase in the expression of CD40. However, JSI-124 was not able to increase the expression of MHC class II, CD86, or CD40 in c-Rel deficient DCs (Fig. 6, E and F). In a separate set of experiments, we confirmed that DCs isolated from *c-Rel*^{-/-} mice indeed respond to 24-h treatment with 5 μ g/ml LPS (Fig. 6E). These data suggest that STAT3 may regulate NF- κ B activation in DCs via direct binding with at least one NF- κ B subunit. Furthermore, the experiments demonstrate that the ability of JSI-124 to induce DC maturation requires a functional c-Rel.

Discussion

In this study we report that Jak2/STAT3 may play a role as a negative regulator of DC activation under normal conditions and in cancer. Previous studies have demonstrated a possible critical role of Jak2/STAT3 hyperactivation in abnormalities observed in DC differentiation in cancer (6, 7). Therefore, inhibition of the Jak/STAT3 pathway may be an attractive therapeutic approach to improve the differentiation and function of DCs in cancer. To test this hypothesis, we used JSI-124, a recently discovered selective JAK/STAT3 signaling pathway inhibitor with potent antitumor activity against human tumors in immune-deficient as well as immune-competent mouse models (10). JSI-124 (cucurbitacin I) is a member of the cucurbitacin family of compounds that are isolated from various plant families, such as the Cucurbitaceae and Cruciferae. A previous study demonstrated that JSI-124 inhibits the cellular levels of phosphotyrosine-STAT3 and phospho-Jak2, but not phospho-ERK1/2, phospho-JNK, or phospho-Akt (10). Importantly, although JSI-124 is very effective at suppressing the levels of tyrosine-phosphorylated STAT3 and Jak2, it is unable to directly inhibit Src kinase or Jak2 activities in vitro, whereas AG-490 (a known inhibitor of Jaks) inhibited the kinase activity of both Jak1 and Jak2 (10). Therefore, JSI-124 could be a good candidate to evaluate the role of Jak2/STAT3 signaling in DCs.

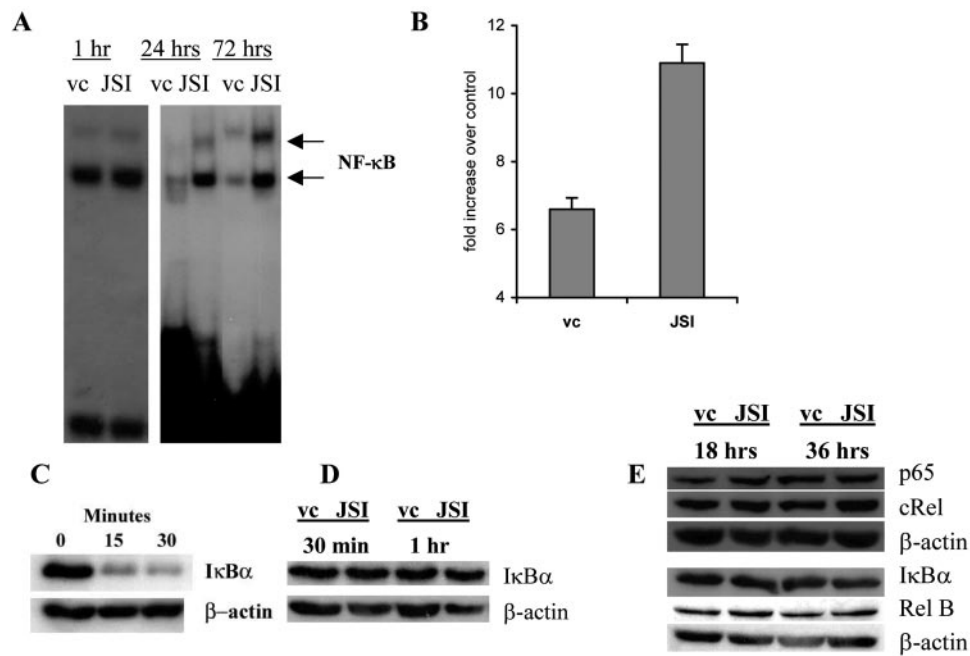


FIGURE 5. Inhibition of Jak2/STAT3 signaling activates NF- κ B in DCs. CD11c⁺ DCs were generated as described in Fig. 4 and treated with either JSI-124 or VC. *A*, DCs were collected at the time points indicated, and EMSA with NF- κ B-specific probe was performed. *B*, CD11c⁺ cells were cotransfected with NF- κ B or control plasmid and plasmid carrying the *Renilla* luciferase gene, then treated with JSI-124 or VC for 36 h. Luciferase activity (LA) values for specific and control plasmid were normalized to *Renilla* LA. The data presented are the fold increase in LA in cells transfected with plasmid containing an NF- κ B-responsive element over the LA in cells transfected with the control plasmid. The mean results of three experiments performed are shown. *C*, DCs were treated with 5 μ g/ml LPS for 15–30 min. The level of I κ B α was evaluated by Western blotting as described in *Materials and Methods*. *D*, Level of I κ B α in DCs after 30 and 60 min of treatment with JSI-124. *E*, DCs were treated with JSI-124 or VC for 18 and 36 h, whole cell lysates were prepared, and the levels of various NF- κ B subunits and I κ B α were determined by Western blotting.

Consistent with previous observations of different groups (reviewed in Ref. 4), TDF significantly inhibited the generation of DCs from HPCs and increased the accumulation of IMCs. JSI-124 substantially reduced the proportion of IMC and increased the presence of DCs. A recent study from Laouar et al. (5) has demonstrated, using conditional knockout mice, that STAT3 is necessary for normal DC differentiation. In other studies, this group reported accumulation of myeloid cells in STAT3-deficient mice (18). We believe that there is no contradiction between our results. In conditional knockout mice, STAT3 was targeted on early stages of myeloid cell differentiation. STAT3 activity in early progenitors is critically important for the development of DCs. In our experiments, STAT3 inhibitor predominantly targeted the population of IMC, which is represented by a mixed group of myeloid cells primarily in the late stages of myeloid cell differentiation. It is likely that the effect of STAT3 on myeloid cells depends on the stage of cell development. At present, the molecular mechanisms of the effect of STAT3 inhibition on myeloid cell differentiation are under investigation.

Most strikingly, inhibition of Jak2/STAT3 caused dramatic activation of DCs. This DC activation resulted in a significant increase in the ability of DC to stimulate an Ag-specific T cell response. These particular findings are not entirely unexpected, because similar observations were made previously using conditional knockout STAT3 mice and the Jak inhibitor of AG490 (6, 15, 18). However, in this study, for the first time, we have clarified the mechanism of this activation. We have demonstrated that the enhanced ability of DCs to stimulate T cells was caused not by increased production of cytokines, but by direct cell-cell contact, apparently due to a dramatic increase in the expression of surface molecules, including MHC class II and costimulatory molecules. There are several potential mechanisms of increased expression of

MHC class II on the surface of DCs induced by JSI-124. First, it is possible that JSI-124 treatment results in increased transcription and biosynthesis of MHC class II molecules. The main factor controlling MHC class II genes is expression of the transcriptional coactivator, CIITA. The gene encoding CIITA (*C2ta* in mice) is controlled by three independent promoters: pI, pIII, and pIV (19); pI is a myeloid promoter driving CIITA and MHC class II expression in macrophages and DCs, whereas pIII is primarily a lymphoid promoter essential for the direction of MHC class II expression in B cells and plasmacytoid DCs (20). JSI-124 completely inhibited the expression of CIITA pI while not affecting CIITA pIII. This is consistent with the classical pathway of myeloid DC differentiation. The second possible mechanism of up-regulation of MHC class II on the surface is translocation of MHC class II molecules from intracellular compartments to the cell surface (21, 22). Our experiments demonstrated the presence of MHC class II molecules inside DCs and their migration to the surface after treatment with JSI-124. Thus, JSI-124 induced DC maturation via a classical pathway that involved loss of the ability to take up soluble Ags, shutdown of MHC class II transcription, and translocation of these molecules on the cell surface.

Because the main mechanism of classical DC maturation involves activation of NF- κ B, we have investigated the effect of STAT3 inhibition on NF- κ B. NF- κ B is present as an inactive complex in the cytoplasm of DCs bound to members of the I κ B family of inhibitory proteins. Activation of NF- κ B involves serine phosphorylation, dissociation, and degradation of I κ B, followed by the release and nuclear translocation of NF- κ B. This activation can be induced by a variety of stimuli, including LPS, TNF, and CD40L (23). Using a DNA binding assay and transcription of the luciferase reporter gene, we found that JSI-124 indeed activated NF- κ B. However, strong NF- κ B activation was not observed during

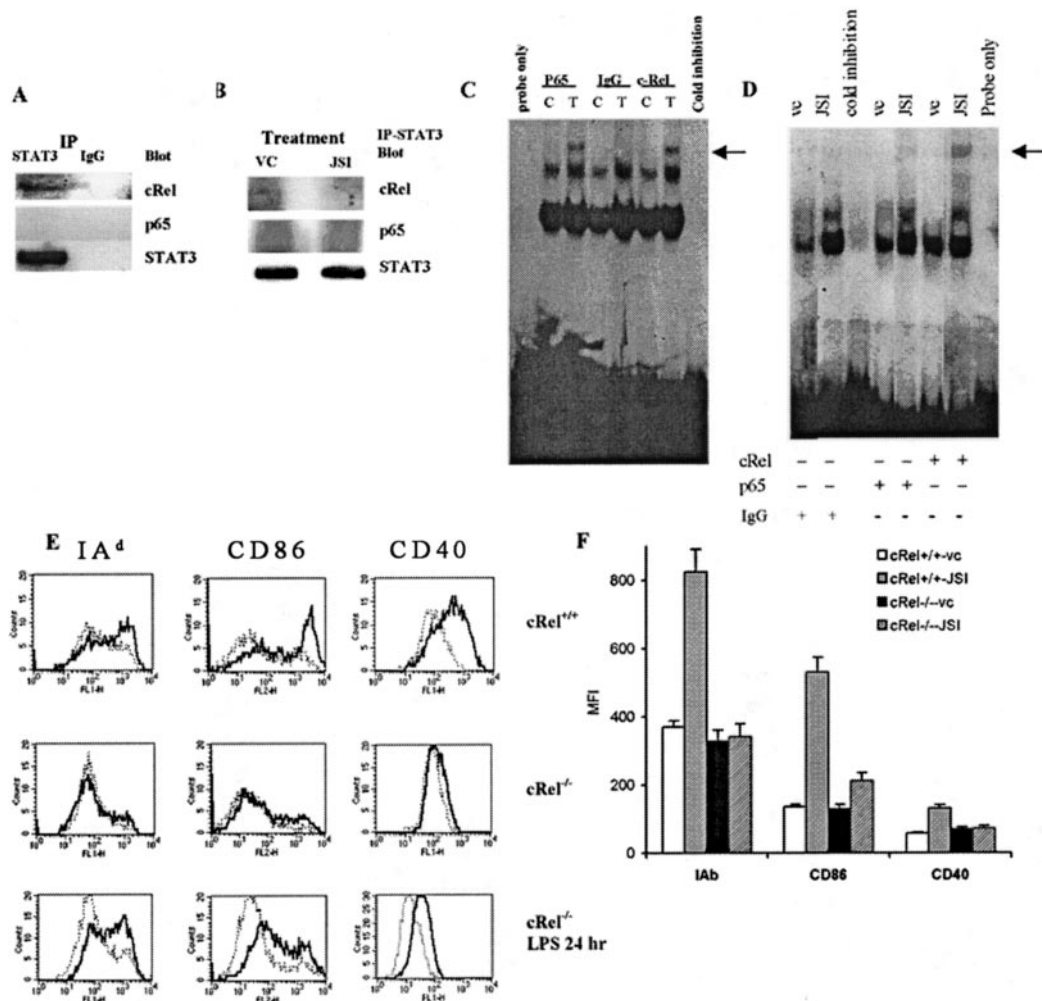


FIGURE 6. Mechanism of JSI-124-induced activation of NF- κ B in DCs. *A*, CD11c⁺ DCs were generated as described above, and cells were stimulated with 20 ng/ml TNF- α for 40 min. Immunoprecipitation was performed with anti-STAT3 Ab or control rabbit IgG as described in *Materials and Methods*. Membranes were then probed with Abs against c-Rel, p65, and STAT3. *B*, CD11c⁺ DCs treated with JSI-124 or VC for 36 h were collected, and cell lysates were prepared. STAT3 was precipitated using anti-STAT3 Ab. Membranes were then probed with Abs against c-Rel, p65, and STAT3. *C*, CD11c⁺ DCs were generated as described in Fig. 4 and treated for 20 min with 20 ng/ml TNF- α (T). In the control (C), cells were cultured in medium alone. Nuclear extracts were prepared, and EMSA was performed with NF- κ B-specific probe in the presence of 5 μ g of rabbit IgG or rabbit polyclonal Abs against p65 or c-Rel (Santa Cruz Biotechnology). The arrow points to the place of the supershift. Cold inhibition, EMSA performed in the presence of a 100-fold excess of unlabeled probe. Probe only, EMSA performed without nucleoproteins. *D*, CD11c⁺ DCs were treated with either JSI-124 or VC for 48 h. Nuclear extracts were prepared, and EMSA was performed as described above. The arrow points to the place of the supershift. *E* and *F*, DCs were generated from BM HPCs obtained from *c-Rel*^{-/-} or control *c-Rel*^{+/+} mice. CD11c⁺ cells were isolated using the magnetic beads separation technique and treated with 0.5 μ M JSI-124 (solid line) or VC (dotted line) for 5 days. Cells were then collected, and the expressions of MHC class II and costimulatory molecules were analyzed by flow cytometry. A typical example (*E*) and cumulative results (*F*) of three experiments performed are shown. In the *bottom row*, labeled *c-Rel*^{-/-} LPS 24 h, DCs generated from *c-Rel*^{-/-} HPCs were isolated using CD11c marker and were immediately activated for 24 h with 5 μ g/ml LPS. In that case, the solid line represents cells treated with LPS.

first 24 h of culture. This activation did not involve degradation of I κ B α or changes in the amount of NF- κ B subunits. This kinetic makes participation of other member of I κ B family unlikely. Several recent studies have suggested a possible direct link between STAT3 and NF- κ B. STAT3 was shown to bind the p65 subunit of NF- κ B and inhibit NF- κ B activity (16). The potential role of a direct interaction between STAT3 and c-Rel was shown in a recent study by Hoentjen et al. (24). In our experiments we observed a weak, but clearly detectable, association between STAT3 and c-Rel. Treatment of DCs with JSI-124 eliminated this association, suggesting that this mechanism may be involved in the effect of JSI-124. To verify the potential role of such an association, we used *c-Rel*^{-/-} mice. Despite lacking one member of the NF- κ B family, these mice had normal development of DCs, and they responded normally to LPS stimulation (17).

We reasoned that if the interaction of STAT3 with c-Rel is important for the JSI-124-mediated effects on DC activation, then in the absence of this protein, the effect of JSI-124 would be reduced. Our experiments confirmed this hypothesis. Control (*c-Rel*^{+/+}) mice responded to JSI-124 by up-regulating MHC class II, B7-2, and CD40, whereas *c-Rel*^{-/-} mice did not. These data suggest a potential mechanism for DC activation after STAT3 inhibition. However, it does not preclude an alternative explanation, such as modification of chromatin by STAT3, suggested in previous studies (24). More studies are needed to clarify the relationship between STAT3 and NF- κ B.

Thus, this study has demonstrated that selective inhibition of the Jak2/STAT3 pathway with the pharmacological agent JSI-124 significantly activates DCs. In this study, for the first time, we have

clarified the possible mechanism of DC activation induced by Jak2/STAT3 inhibition and suggested that pharmacological inhibition of the Jak2/STAT3 pathway may be potentially useful in cancer immunotherapy. This study was focused on the mechanism of the effects of Jak2/STAT3 inhibition on DCs. Verification of potential use of this approach in cancer immunotherapy will require more vigorous analysis *in vivo*.

Disclosures

The authors have no financial conflict of interest.

References

- Steinman, R. M. 2003. Some interfaces of dendritic cell biology. *APMIS* 111: 675–697.
- Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. A. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18: 767–811.
- Lanzavecchia, A., and F. Sallusto. 2001. Regulation of T cell immunity by dendritic cells. *Cell* 106: 263–266.
- Gabrilovich, D. 2004. The mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat. Rev. Immunol.* 4: 941–952.
- Laouar, Y., T. Welte, X.-Y. Fu, and R. A. Flavell. 2003. STAT3 is required for Flt3L-dependent dendritic cell differentiation. *Immunity* 19: 903–912.
- Wang, T., G. Niu, M. Kortylewski, L. Burdelya, K. Shain, S. Zhang, R. Bhattacharya, D. Gabrilovich, R. Heller, D. Coppola, et al. 2004. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat. Med.* 10: 48–54.
- Nefedova, Y., M. Huang, S. Kusmartsev, R. Bhattacharya, P. Cheng, R. Salup, R. Jove, and D. Gabrilovich. 2004. Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer. *J. Immunol.* 172: 464–474.
- Rane, S. G., and E. S. Reddy. 2000. Janus kinases: components of multiple signaling pathways. *Oncogene* 19: 5662–5679.
- Imada, K., and W. J. Leonard. 2000. The JAK-STAT pathway. *Mol. Immunol.* 37: 1–11.
- Blaskovich, M. A., J. Sun, A. Cantor, J. Turkson, R. Jove, and S. M. Sebti. 2003. Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. *Cancer Res.* 63: 1270–1279.
- Cheng, P., A. Zlobin, V. Volgina, S. Gottipati, B. Osborne, L. Miele, and D. I. Gabrilovich. 2001. Notch-1 regulates NF- κ B activity in hematopoietic progenitor cells. *J. Immunol.* 167: 4458–4467.
- Gabrilovich, D. I., H. L. Chen, K. R. Girgis, T. Cunningham, G. M. Meny, S. Nadaf, D. Kavanaugh, and D. P. Carbone. 1996. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat. Med.* 2: 1096–1103.
- Kusmartsev, S., F. Cheng, B. Yu, Y. Nefedova, E. Sotomayor, R. Lush, and D. I. Gabrilovich. 2003. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. *Cancer Res.* 63: 4441–4449.
- Pisarev, V., B. Yu, R. Salup, S. Sherman, D. C. Altieri, and D. I. Gabrilovich. 2003. Full-length dominant-negative survivin for cancer immunotherapy. *Clin. Cancer Res.* 15: 6523–6533.
- Cheng, F., H. W. Wang, A. Cuenca, M. Huang, T. Ghansah, J. Brayer, W. G. Kerr, K. Takeda, S. Akira, S. P. Schoenberger, et al. 2003. A critical role for Stat3 signaling in immune tolerance. *Immunity* 19: 425–436.
- Yu, Z., W. Zhang, and B. C. Kone. 2002. Signal transducers and activators of transcription 3 (STAT3) inhibits transcription of the inducible nitric oxide synthase gene by interacting with nuclear factor κ B. *Biochem. J.* 367: 97–105.
- Ouaaz, F., J. Arron, Y. Zheng, Y. Choi, and A. A. Beg. 2002. Dendritic cell development and survival require distinct NF- κ B subunits. *Immunity* 16: 257–270.
- Welte, T., S. S. Zhang, T. Wang, Z. Zhang, D. G. Hesslein, Z. Yin, A. Kano, Y. Iwamoto, E. Li, J. E. Craft, et al. 2003. STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. *Proc. Natl. Acad. Sci. USA* 100: 1879–1884.
- Reith, W., and B. Mach. 2001. The bare lymphocyte syndrome and the regulation of MHC expression. *Annu. Rev. Immunol.* 19: 331–373.
- LeibundGut-Landmann, S., J. M. Waldburger, C. Reis e Sousa, H. Acha-Orbea, and W. Reith. 2004. MHC class II expression is differentially regulated in plasmacytoid and conventional dendritic cells. *Nat. Immunol.* 5: 899–908.
- Pierre, P., S. J. Turley, E. Gatti, M. Hull, J. Meltzer, A. Mirza, K. Inaba, R. M. Steinman, and I. Mellman. 1997. Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* 388: 787–792.
- Turley, S. J., K. Inaba, W. S. Garrett, M. Ebersold, J. Unternaehrer, R. M. Steinman, and I. Mellman. 2000. Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 288: 522–527.
- Liou, H. C., and D. Baltimore. 1993. Regulation of the NF- κ B/rel transcription factor and I κ B inhibitor system. *Curr. Opin. Cell Biol.* 5: 477–487.
- Hoentjen, F., R. B. Sartor, M. Ozaki, and C. Jobin. 2005. STAT3 regulates NF- κ B recruitment to the IL-12p40 promoter in dendritic cells. *Blood* 105: 689–696.