

Protein kinase CK2 controls T-cell polarization through dendritic cell activation in response to contact sensitizers

Marie de Bourayne,* Yann Gallais,* Zeina El Ali,*¹ Philippe Rousseau,*²
Marie-Hélène Damiens,* Claude Cochet,[†] Odile Filhol,[†] Sylvie Chollet-Martin,* Marc Pallardy,*
and Saadia Kerdine-Römer*³

*UMR996, Inflammation, Chemokines, and Immunopathology, INSERM, University Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, France; and, [†]Cancer and Infection Biology, UMR1036, INSERM Institute of Life Sciences Research and Technology (iRTSV), Atomic Energy and Alternative Energy Commission (CEA), Grenoble, France

RECEIVED JULY 28, 2015; REVISED SEPTEMBER 2, 2016; ACCEPTED SEPTEMBER 6, 2016. DOI: 10.1189/jlb.3A0715-320RR

ABSTRACT

Allergic contact dermatitis (ACD) represents a severe health problem with increasing worldwide prevalence. It is a T-cell-mediated inflammatory skin disease caused by chemicals present in the daily or professional environment. NiSO₄ and 2,4-dinitrochlorobenzene (DNCB) are 2 chemicals involved in ACD. These contact sensitizers are known to induce an up-regulation of phenotypic markers and cytokine secretion in dendritic cells (DCs; professional APCs), leading to the generation of CD8⁺ Tc1/Tc17 and CD4⁺ Th1/Th17 effector T cells. In the present study, using a peptide array approach, we identified protein kinase CK2 as a novel kinase involved in the activation of human monocyte-derived DCs (MoDCs) in response to NiSO₄ and DNCB. Inhibition of CK2 activity in MoDCs led to an altered mature phenotype with lower expression of CD54, PDL-1, CD86, and CD40 in response to NiSO₄ or DNCB. CK2 activity also regulated proinflammatory cytokine production, such as TNF- α , IL-1 β , and IL-23 in MoDCs. Moreover, in a DC/T cell coculture model in an allogeneic setup, CK2 activity in MoDCs played a major role in Th1 polarization in response to NiSO₄ and DNCB. CK2 inhibition in MoDCs led to an enhanced Th2 polarization in the absence of contact sensitizer stimulation. *J. Leukoc. Biol.* 101: 703–715; 2017.

Introduction

ACD is a T-cell-mediated inflammatory skin disease caused by type IV delayed hypersensitivity responses to contact allergens [1]. Contact allergens are chemically reactive haptens

(molecular mass, <500 Da). During skin penetration, they bind to self-proteins to generate immunogenic neoantigens [2]. DCs are the major players in the sensitization phase, the first phase of ACD. This phase is clinically unapparent; it occurs at the first contact of skin with the chemical sensitizer and leads to the generation of hapten-specific T cells [3]. DCs capture the hapten-self-protein complex, migrate through the lymphatic vessels, and present the hapten-protein complex to naive T cells in the draining lymph node to generate CD8⁺ Tc1/Tc17 and CD4⁺ Th1/Th17 effector T cells, which enter the blood circulation [3, 4]. The second phase of ACD, termed the elicitation phase, occurs upon re-exposure of sensitized individuals to the same hapten, and the reaction becomes clinically apparent. Specific T lymphocytes are activated in the dermis and the epidermis and trigger the inflammatory process responsible for cutaneous lesions [3].

More than 3000 contact allergens have been identified. Nickel, present in jewelry, piercing jewelry, coins, and smartphones, is a metallic hapten ranked as the number one allergen in frequency of positive patch test reactions [5]. DNCB is used as a reactive intermediate during the manufacture of fungicides, dyes, and elastomers. It is used to study the mechanisms of contact sensitivity, because it can induce ACD in all immunocompetent humans [6]. Moreover, DNCB is still the reference product in regulatory tests to measure the potency of sensitization.

In human in vitro DC models, we and others have demonstrated that chemical sensitizers such as NiSO₄ induce phenotypic modifications, with HLA-DR, CD86, CD40, CD83, and CCR7 up-regulation and secretion of inflammatory cytokines,

Abbreviations: ACD = allergic contact dermatitis, CK2 = protein kinase CK2 (formerly casein kinase 2), cMFI = corrected MFI, CX-4945 = 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid, DC = dendritic cell, DNA-PKC = DNA dependant protein kinase C, DNCB = 2,4-dinitrochlorobenzene, IRF = interferon regulatory factor, MFI = mean fluorescence intensity, MoDC = monocyte-derived dendritic cell, MSD = Meso Scale Discovery (assay), PDL-1 = programmed death ligand-1, RFI = relative fluorescence intensity, RPMIc = complete RPMI medium

1. Current affiliation: Mondor Institute of Biomedical Research (IMRB), UMR-S955, Team 12, Université Paris-Est, Créteil, France.
2. Current affiliation: Bloomfield Center for Research in Aging, Lady Davis Institute for Medical Research, Jewish General Hospital, Montréal, Québec, Canada.
3. Correspondence: INSERM UMR-996; Univ. Paris-Sud Univ. Paris Saclay, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, F-92296 Châtenay-Malabry, E-mail: saadia.kerdine-romer@u-psud.fr

such as IL-8 and TNF α [7–10]. Signaling pathways, such as MAPK and NF- κ B, have been reported to be involved in DC maturation in response to contact sensitizers [8, 9, 11, 12]. Thus, inhibition of p38 MAPK, JNK, and ERK abrogate the NiSO $_4$ -induced up-regulation of CD86, CD83, and CCR7 expression. Otherwise, it has been demonstrated that p38 MAPK, NF- κ B, and IRF-1 are involved in IL-12p40 production induced by NiSO $_4$ [13]. In response to DNCB, p38 MAPK controls the expression of HLA-DR, CD86, and CD83 and the production of IL-8 by DCs [9]. Moreover, p38 MAPK and JNK control TNF- α release in DNCB-stimulated DCs [7].

The hypothesis that chemical sensitizers can be perceived as a danger signal by DCs has also been proposed, based on signaling pathways identified by chemical sensitizer treatment, and is known to be triggered by danger signals such as TLR agonists [7, 8, 12]. Signaling pathways, such as MAPKs and NF- κ B, are known to be redox sensitive, and oxidative stress induced by chemical sensitizers has been shown to participate in the DC-maturation process (for review, see ref. 14). However, very little is known regarding upstream signals, and therefore pathways that could be involved in MAPK and NF- κ B activation remain to be found. To this end, we used a peptide array to find the original pathways implicated in the biologic response of DCs to contact sensitizers. Results from this peptide array analysis have highlighted the activation of CK2 (formerly casein kinase 2), a multifunctional and pleiotropic protein that is ubiquitous and constitutively active. CK2 is a multisubunit protein kinase that exhibits a quaternary structure composed of 2 catalytic subunits, CK2 α and CK2 α' , and 2 regulatory subunits, CK2 β . As a signaling protein, CK2 is a prodigious kinase having many cellular functions associated with a wide repertoire of substrates. Indeed, CK2 has been found to regulate key transcription factors implicated in inflammation and cytokine signaling, such as NF- κ B and AP-1 [15]. The biologic importance of CK2 in diseases such as cancer has led to the development of an orally available small molecule inhibitor, CX-4945 [16].

To investigate the role of CK2 in DC activation in response to contact sensitizers, we used a highly specific pharmacological inhibitor of CK2, CX-4945, which displays high potency and an unequaled selectivity for CK2 [17].

In DCs differentiated from human MoDCs, we showed that CK2 (1) plays a role in phenotypic maturation and cytokine production in response to NiSO $_4$ and DNCB and (2) has a major role in CD4 $^+$ T-lymphocyte activation and in the control of Th1 polarization without affecting Th17 polarization.

MATERIALS AND METHODS

Generation of DCs derived from human MoDCs

The generation of human MoDCs was performed as described elsewhere [13]. In brief, PBMCs were isolated from whole blood of healthy donors obtained from Etablissement Français du Sang (EFS, Rennes, France) by density centrifugation with Ficoll-gradient (lymphocyte separation medium LSM 1077; PAA, Les Mureaux, France). Monocytes were further isolated from the mononuclear fraction through magnetic positive selection by using MiniMacs separation columns (Miltenyi Biotec, Bergish Gladbach, Germany), and anti-CD14 antibodies were coated on magnetic beads according to the manufacturer's instructions (direct CD14 isolation kit; Miltenyi Biotec). These CD14 $^+$ cells have been confirmed to have purity greater than 95%, based on

CD14 expression (555398; BD Biosciences, Le Pont de Claix, France) evaluated by flow cytometry (FACSCalibur; BD Biosciences). Monocytes were cultured at 1×10^6 cells/ml in the presence of GM-CSF (550 U/ml) and IL-4 (550 U/ml) (both from Miltenyi Biotec) in RPMI 1640 medium with Glutamax and 25 mM HEPES (ThermoFisher Scientific, Carlsbad, California, USA) supplemented with 10% heat-inactivated FBS (Biowest, Paris, France), 1% sodium pyruvate (ThermoFisher Scientific), and 1% penicillin/streptomycin (ThermoFisher Scientific) [RPMIc] for 5 d. The resulting MoDCs were DC-SIGN $^{\text{high}}$, CD1a $^{\text{high}}$, CD86 $^{\text{low}}$, and CD83 $^{\text{low}}$, as evaluated by flow cytometry (FACSCalibur; BD Biosciences).

Chemical treatment

MoDCs were washed twice and then incubated at 1×10^6 cells/ml. The cells were treated with DNCB (10 μ M) or NiSO $_4$ (500 μ M; both from Sigma-Aldrich, St. Louis, MO, USA) for different periods, depending on the experiment. DNCB was dissolved in DMSO at 0.1%, as a final concentration in complete medium, whereas NiSO $_4$ was dissolved in saline. Inhibition of CK2 was performed with the pharmacological inhibitor CX-4945 (5 μ M; Santa Cruz Biotechnology, Heidelberg, Germany).

Phosphokinome analysis of NiSO $_4$ - and DNCB-stimulated MoDCs

Human MoDCs from 10 healthy donors were selected for their response to NiSO $_4$ and DNCB: CD83 and CD86 up-regulation at 24 h determined by flow cytometry (described below) and activation of phospho-p38 at 30 min, evaluated by Western blot.

Immature MoDCs (1×10^6 cells/donor/chemical) were treated with vehicle (DMSO or saline), NiSO $_4$ (500 μ M), or DNCB (10 μ M) for 30 or 60 min in culture conditions (37°C, 5% CO $_2$) and were collected on ice, washed with PBS, and resuspended in 25 μ l of the cold lysis buffer Triton 1% [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Na $_4$ P $_2$ O $_4$, 1 mM β -glycerophosphate, 1 mM Na $_3$ VO $_3$, and 1 mM NaF, supplemented with Complete protease inhibitor cocktail (Roche Diagnostics, Meylan, France)]. Cell lysates were pooled by treatment (molecule/time), cleaned by centrifugation, diluted in buffer [50 mM HEPES-KOH (pH 7.5), 10 mM MgCl $_2$, 10 mM MnCl $_2$ supplemented with Complete protease inhibitor cocktail (Roche)], and cleaned a second time, as above. Supernatant (80 μ l) was mixed with 10 μ l Activation Mix [20 μ Ci of γ ^{33} P-ATP (GE Healthcare Life Sciences, Velizy-Villacoublay, France), 50% glycerol, 5 mM DTT, 50 mM MgCl $_2$, 50 mM MnCl $_2$, 250 μ g/ml PEG 8000, and 250 μ g/ml BSA], cleared by an additional centrifugation, loaded on 1 PepChip Kinomics Array (Pepscan Presto, Lelystad, The Netherlands) per sample, and incubated for 2 h at 37°C in a humidified chamber. Arrays were washed 4 times alternatively with 2 M NaCl 1% Triton X-100 solution and PBS 1% Triton X-100 solution, rinsed 3 times with distilled water, air dried, and exposed on a phosphor storage screen (GE Healthcare Life Sciences). The screen was then scanned with a resolution of 50 μ m/pixel on a Storm Imager (GE Healthcare Life Sciences), and the array scans were quantified with in-house developed quantification software in a MatLab (Natick, MA, USA) environment (Pepscan Presto). Arrays contain 1024 peptides (48 controls, 976 experimental, representing redundant phosphorylatable motifs identified in the human genome), each in triplicate on an array. One array per sample resulted in a maximum of 3 data points per peptide. Background was subtracted for all individual spots. The spots were corrected for local minima and maxima on the array and flagged for the number of artifacts. The spots were subsequently filtered with the Kolmogorov-Smirnov statistic at a cutoff of $P = 0.001$, to exclude those with a value distribution that could not be distinguished from the background, and a minimum of 2 values per peptide. As a threshold for significance, only ratios corresponding to mean differences of $2.0 \times (\text{SEM } 1 + \text{SEM } 2)$ were considered.

CK2 activity assay

The measurement of CK2 activity was performed by using a published procedure [18]. In brief, reaction was started by addition of the sample (3 μ l)

to the reaction mixture [20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 150 mM NaCl, 30 μM ATP, 45 μM of the synthetic peptide RRREDEESDDEE, and 1 μCi of γ-[³²P]-ATP/reaction in a final volume of 20 μl]. The reaction was performed for 5 min at room temperature and stopped with 60 μl of 4% cold TCA. After centrifugation of the reaction mixture, the supernatant was spotted on phosphocellulose paper squares. After 3 successive 15 min washes in cold phosphoric acid (0.5%), the radioactivity on phosphocellulose paper was counted in scintillation liquid. Results are expressed either as total activity (cpm) or as specific activity (cpm/milligram protein per minute of reaction). Protein concentration was determined with the micro-bicinchoninic acid technique.

Treatment of MoDCs

On d 5, immature MoDCs were harvested and washed in RPMIc. MoDCs were then pretreated with CX-4945, the specific pharmacological inhibitor of CK2 (5 μM, sc-364475; Santa Cruz Biotechnology). After 1 h of incubation at 37°C, MoDCs were treated or not with NiSO₄ (500 μM) or DNCB (10 μM). The stimulation time at 37°C ranged from 30 min to 24 h, depending on the experiment.

Functional analysis of MoDC endocytosis: internalization of dextran-FITC

The endocytic capacity of the cells was analyzed by the measure of dextran-FITC uptake, a probe for mannose receptor-mediated endocytosis. MoDCs were pretreated for 1 h at 37°C with DMSO or CX-4945 (5 μM) and then stimulated or not with NiSO₄ (500 μM) or DNCB (10 μM) for 24 h, washed, and resuspended in RPMIc at 2×10^6 cells/ml. One hundred microliters of the cell suspension was incubated for 1 min at 37°C (or at 4°C for controls) before the addition of 100 μl of 1 mg/ml dextran-FITC (molecular mass, 40 kDa; ThermoFisher Scientific) solution for different times. At 15 min, 30 min, 1 h, or 2 h, the reaction was stopped by adding 4 ml cold PBS; then, the cells were washed 4 times with cold PBS. The uptake of dextran-FITC was analyzed by flow cytometry with a FACSCalibur cell analyzer and CellQuest software (BD Biosciences).

Flow cytometric analysis of MoDC maturation

After 24 h of treatment with DMSO or CX-4945 with or without chemical sensitizers (NiSO₄ or DNCB), the MoDCs phenotype was analyzed by flow cytometry. The labeling procedure was performed as follows: 2×10^5 cells/ml were washed in cold PBS supplemented with 0.5% of BSA and stained with mAbs in the dark on ice for 20 min. After a wash with cold PBS, the cells are fixed in 1% paraformaldehyde diluted in PBS. The following mAbs were used: FITC-labeled mouse anti-human CD86 [2331 (FUN-1); BD Biosciences], anti-human CD40 (5C3; BD Biosciences) and anti-human PDL-1 (CD274; MIH1; BD Biosciences); allophycocyanin-conjugated mouse anti-human CD83 (HB15e; BD Biosciences), anti-human CD54 (HA58; BD Biosciences), and anti-human HLA-DR (G46-6; BD Biosciences). Appropriate isotype controls (mouse IgG1κ; BD Biosciences) were used at the same concentration to determine nonspecific staining. A minimum of 10,000 cells were analyzed on a FACSCalibur cell analyzer with CellQuest software (BD Biosciences). Living cells were identified on the basis of their physical characteristics [forward scatter and side scatter]. Results are expressed as the RFI, using the cMFI, as follows: cMFI = MFI of cells/MFI of isotype control; RFI = cMFI of treated cells/cMFI of untreated cells.

Quantitative real-time RT-PCR assay of cytokines in MoDCs

Total RNA was extracted after lysis of MoDCs treated for 6 h (10^6 cells/ml) with RNA-PLUS reagent (MP Biomedicals, Santa Ana, CA, USA). Total RNA pellets were resuspended in RNase-free water and quantified by spectrophotometry. First-strand cDNA was synthesized from total RNA on a thermocycler (Biometra, Göttingen, Germany). The reaction used 1 μg of total RNA, a dNTP mixture (containing 25 mM dATP, dGTP, dCTP, and dTTP) and 50 μM oligo (dT) primers (MWG Biotech, Ebersberg, Germany). Reverse

transcription was performed in 1× AMV reverse transcriptase reaction buffer (Promega, Charbonnières-les-Bains, France), with RNase inhibitor (RNasin; Promega) at 40 U/μl, AMV reverse transcriptase (Promega) at 10 U/μl, and RNase-free water, to a final volume of 10 μl. A control without reverse transcriptase was used to confirm the absence of DNA contamination. Real-time PCR was performed with SYBR Green technology on a CFX96 system (Bio-Rad, Marnes-la-Coquette, France). Each reaction mix consisted of 1:50 diluted cDNA in a 4 μl final volume of nuclease-free water; 0.5 μM of each forward and reverse primer for *il-12p35*, *il-12p40*, *il-23p19*, *irf-1*, *irf-4*, *irf-8*, *Gapdh*, and *b-actin*; and Sso Advanced Supermix (Bio-Rad) in a total reaction volume of 10 μl. The following specific primers are used (forward and reverse, respectively): *il-12p40*: 5'-TGG AGT GCC AGG AGG ACA GT-3' and 5'-TCT TGG GTG GGT CAG GTT TG-3'; *il-12p35*: 5'-ACC ACT CCC AAA ACC TGC-3' and 5'-CCA GGC AAC TCC CAT TAG-3'; *il-23p19*: 5'-GTT CCC CAT ATC CAG TGT GG-3' and 5'-TCC TTT GCA AGC AGA ACT GA-3'; *irf-1*: 5'-ACC CTG GCT AGA GAT GCA GA-3' and 5'-TTT TCC CCT GCT TGT ATC G-3'; *irf-4*: 5'-AGC ATC TTC CGC ATC CCC-3' and 5'-CAT CTG CGG GTC CTC CAA-3'; *irf-8*: 5'-GAC AGT GGC TGA TCG AGC AG-3' and 5'-CTT CCA AGT GGC TGG TTC AGC-3'; *gapdh*: 5'-CAG CCT CAA GAT CAG CAG CA-3' and 5'-TGT GGT CAT GAG TCC TTC CA-3'; and *b-actin*: 5'-GGC ATC CTC ACC CTG AAG TA-3' and 5'-GCA CAC GCA GCT CAT TGT AG-3'.

After 30 s at 95°C for Sso7d fusion polymerase activation, amplification was allowed to proceed for 44 cycles, each consisting of denaturation at 95°C for 5 s and annealing/extension at 62°C for 5 s. Eightfold serial dilutions of mixed cDNA (from different samples) were analyzed for each target gene, allowing us to construct linear standard curves from which the efficiency (*E*) of each PCR run was evaluated. SYBR green fluorescence was detected at the end of each elongation cycle, after which a melting curve was constructed to confirm the specificity of the PCR products. Quantification was performed with CFX Manager Software (Bio-Rad), and data were analyzed by the $\Delta\Delta C_t$ method. Ratios are calculated as the geometric mean of $(1 + E) - \Delta\Delta C_t$, where *E* is the efficiency and $\Delta\Delta C_t$ is the target gene expression of treated cells compared with normal levels in untreated cells, with correction for the expression of the reference genes *b-actin* and *GAPDH*. Results are expressed as the fold factor increase (*i.e.*, ratio of $(1 + E) - \Delta\Delta C_t$ of treated cells/ $(1 + E) - \Delta\Delta C_t$ of untreated cells).

T-lymphocyte proliferation

The capacity of MoDCs to activate CD4⁺ T cells was evaluated by the use of allogeneic T CD4⁺ lymphocytes labeled with 0.5 μM CFSE, according to the manufacturer's instructions. CD4⁺ T cells were isolated from PBMCs by positive selection with the MACS CD4 isolation kit II (Miltenyi Biotec). These T cells were confirmed to have purity greater than 95%, based on CD4 (555349; BD Biosciences) expression evaluated by flow cytometry.

MoDCs were pretreated or not with CX-4945 (5 μM) and then stimulated with NiSO₄ or DNCB for 24 h. Treated MoDCs were washed and then cultured with allogeneic CD4⁺ T cells at a 1:10 DC/T-cell ratio for 5 d in RPMI 1640 Glutamax supplemented with 10% AB human serum, in round-bottomed 96-well plates. On day 5, CD4⁺ T-cell proliferation was analyzed by flow cytometry and expressed as the percentage of CFSE^{low} cells present in the sample. Cell division was next determined by analyzing CFSE dilution with a FACSCalibur flow cytometer and CellQuest software (BD Biosciences). Supernatants were then analyzed for cytokine release, as described below.

T-lymphocyte polarization in response to MoDCs

To study differentiation of naive Th cells into Th1, Th2, or Th17 effectors cells, naive CD4⁺ T cells, isolated using naive CD4⁺ T cell isolation kit II (130-094-131, Miltenyi Biotec), were cocultured with treated MoDCs. After 5 d of coculture, the T cells were stimulated with anti-CD2/3/28 beads (bead-to-cell ratio 1:2) from a T-cell activation and expansion kit (130-091-441, Miltenyi Biotec) for 48 h. Supernatants were analyzed for cytokine release (as described below), and the cells were stained with anti-CD4 Alexa Fluor 700 (56-0049-42; eBioscience, Paris, France), fixed, and permeabilized with a Foxp3 staining buffer set (00-5523, eBioscience), according to the manufacturer's instructions. For other transcriptional factors, RORγT-APC

(17-6988-82; eBioscience), GATA3-PE (12-996642, eBioscience), and T-Bet-Brilliant Violet 605 (644817, BioLegend, London, UK) were used for staining. The cells were analyzed on an FACS Fortessa with Diva software (BD Biosciences) and gated on living cells based on ZombieAqua staining (BioLegend).

Cytokine quantification in MoDCs and MoDC/T-cell culture supernatants

The supernatants of 24 h cultured MoDCs were assayed for IL-6, IL-8, TNF- α , IL-1 β , IL-12p70, IL-23, and MIP-1 β production, with an MSD multiplex assay (Human Proinflammatory Panel-1, Prototype Human IL-23, and Chemokine Panel-1 kits; Meso Scale Discovery, Gaithersburg, MD, USA).

Supernatants of MoDC-naïve or total CD4⁺ T cell cocultures were also analyzed for their cytokine production. IFN- γ , IL-5, IL-13, IL-10, and IL-17 (Human Proinflammatory Panel-1 and V-PLEX Custom Human Cytokine Panel-1 kits) were quantified with the MSD multiplex assay.

Statistical analysis

Data are expressed as means $\pm$ SEM. Differences between groups were evaluated with the Mann-Whitney *U* test (Prism Software; GraphPad, La Jolla, CA, USA). *P* < 0.05 was regarded as significant.

RESULTS

Phosphokinome array

MoDCs from donors exhibiting at least a 1.5-fold increase in CD86 and phosphorylation of p38 MAPK at 30 and 60 min in response to chemical sensitizers were analyzed. As many upstream kinases were activated in response to the chemicals, only the top 5 up-regulated kinases were presented for each time and each chemical (Fig. 1).

DNCB and NiSO₄ activated different kinase profiles that are important in the DCs' biologic process (Fig. 1). We found that 2 of these kinases were highly activated by both chemicals at both times (30 and 60 min): ERK and CK2 subunits α or α' . Furthermore, cell division cycle protein 2 homolog, PKC, JNK, DNA-dependent protein kinase C, and Fyn were highly activated but have already been described as key players in DCs activation and function, such as ERK [19–23]. Among all these kinases, only CK2 was a novel kinase that had not been described to play a role in DC biology to date. Thus, we chose to evaluate the role of CK2 in NiSO₄- and DNCB-stimulated MoDCs.

Inhibition of CK2 activity by CX-4945 does not modulate the uptake of dextran-FITC by MoDCs

To address the role of CK2, we used a specific pharmacologic inhibitor of CK2, CX-4945, a potent small inhibitor of CK2 displaying an excellent kinase selectivity profile [24] and drastically inhibiting CK2 activity at 5 μ M (Fig. 2A), without cytotoxicity in MoDCs (data not shown). Our results showed that global CK2 activity was not modulated by the chemical treatment, contrary to the results observed in the phosphokinome, which measured the activity on specific phosphorylation motives depending on the substrate (Fig. 1). This result was not surprising, because CK2 is constitutively active [15].

DCs have 3 main functions: endocytosis, migration, and antigen presentation to T lymphocytes. First, we examined whether the inhibition of CK2 activity by CX-4945 impairs

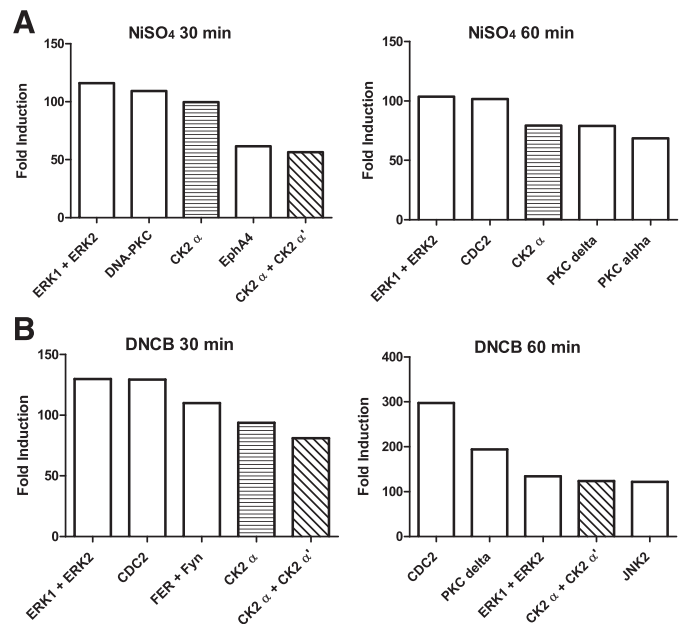


Figure 1. MoDCs display important kinase activity in response to NiSO₄ and DNCB. Peptide microarray analysis of the predicted kinase activity of the top 5 kinases up-regulated in MoDCs by 30 and 60 min stimulation with NiSO₄ 500 μ M (A) or DNCB 10 μ M (B). Protein lysates of 10 independent donors were pooled and results were expressed as fold increase compared to control. DNA-PKC: DNA dependant kinase C; EphA4: ephrin type-A receptor 4; CDC2: cell division cycle protein 2 homolog; FER: (fps/fes related) tyrosine kinase.

endocytic capacity, analyzed by dextran-FITC uptake by immature MoDCs. Cells were thus pretreated or not with CX-4945 for 1 h, washed, and incubated with dextran-FITC solution at 37°C for different times (Fig. 2B). MoDCs' endocytic capacity was identical in the presence or absence of CX-4945. As the negative control, similar experiments are performed at 4°C, in which the uptake of dextran-FITC by MoDCs was inhibited. As expected, mature MoDCs (MoDCs stimulated for 24 h with NiSO₄ or DNCB) had a decreased ability to take up dextran-FITC at 30 min. CX-4945 pretreatment did not modulate the dextran-FITC uptake in stimulated MoDCs (Fig. 2C). These data indicate that CK2 does not control endocytic activity in stimulated and unstimulated MoDCs.

Inhibition of CK2 activity down-regulates the expression of key surface markers for MoDCs maturation

Because MoDCs activated by contact sensitizers have been shown to acquire a mature phenotype, we evaluated the role of CK2 in the acquisition of a mature phenotype in response to NiSO₄ or DNCB. After incubation with CX-4945 for 1 h, MoDCs were stimulated for 24 h with NiSO₄ or DNCB, and the expression of several MoDC maturation markers was quantified by flow cytometry. As previously described, NiSO₄ and DNCB induced MoDC maturation [8, 9, 25], as shown by the increased expression of CD86, CD83, CD40, PDL-1, and MHC II (Fig. 3A and B). Contrary to NiSO₄, DNCB did not increase CD54 expression in our hands.

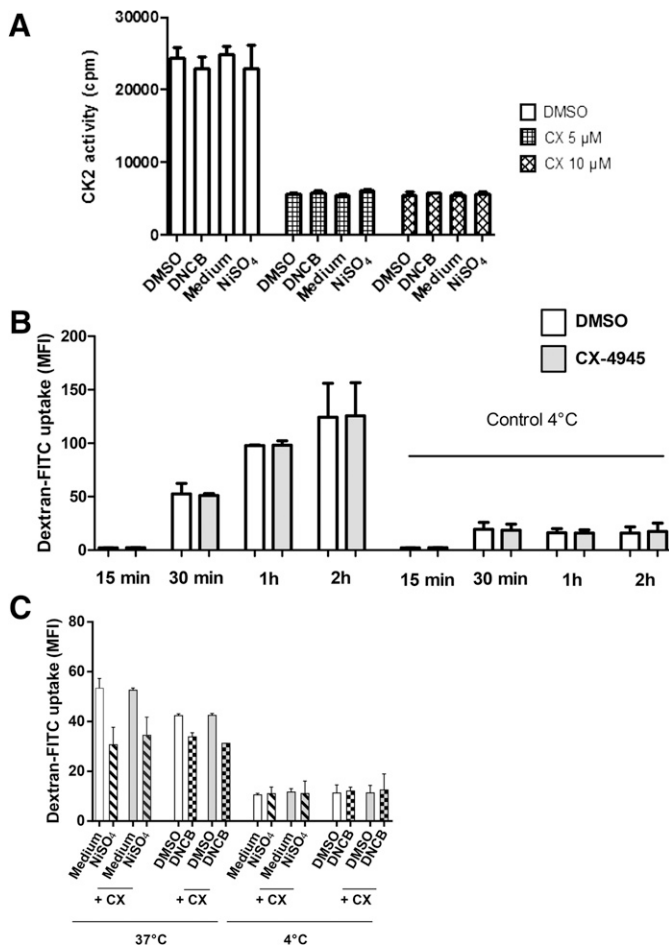


Figure 2. Inhibition of CK2 activity does not modulate uptake of dextran-FITC by MoDCs. MoDCs were pretreated for 1 h with CX-4945 (CX, 5 or 10 μ M) and stimulated for 6 h with NiSO₄ (500 μ M) or DNCB (10 μ M). CK2 activity was measured by incorporation of γ -[³²P]-ATP on a synthetic peptide specific to CK2 (A). Results are expressed as total activity (cpm) and are representative of 3 independent experiments. MoDCs were incubated with DMSO 0.1% or CX-4945 (CX, 5 μ M) for 1 h before incubation with dextran-FITC (0.5 mg/ml) at 37°C for various times and the uptake of dextran-FITC was analyzed by flow cytometry (B). MoDCs were pretreated for 1 h with DMSO or CX-4945 (5 μ M) and stimulated or not with NiSO₄ (500 μ M) and DNCB (10 μ M) for 24 h before incubation with dextran-FITC (0.5 mg/ml) at 37°C for 30 min (C). MoDCs incubated with dextran-FITC (0.5 mg/ml) at 4°C were used as the control. Histogram represents MFI of 3 independent experiments.

Pretreatment of MoDCs with CX-4945 significantly decreased NiSO₄-induced up-regulation of CD86, PDL-1, and CD54 expression, with the following percentage reductions: CD86, 44 $\pm$ 7%; PDL-1, 60 $\pm$ 8%; and CD54, 36 $\pm$ 7%. Only a slight decrease was observed in CD83 and CD40 (Fig. 3A). Pretreatment of DNCB-stimulated MoDCs with CX-4945 significantly decreased PDL-1, CD54, and CD40 expression, with the following percentage reductions: PDL-1, 38 $\pm$ 8%; CD54, 57 $\pm$ 10%; and CD40: 40 $\pm$ 8% (Fig. 3B). In control cells, CK2 did not modulate the expression of the studied markers, CD86, CD83, PDL-1, CD54, and CD40, but negatively controlled basal

MHC II expression. Indeed, control cells pretreated with CX-4945 exhibited a significant increase in MHC II expression (Fig. 3A and B).

These results show that CK2 controlled PDL-1 and CD54 expression in response to DNCB and NiSO₄, whereas CK2 controlled CD86 expression in response to NiSO₄ and CD40 in response to DNCB. Taken together, these data demonstrate that the inhibition of CK2 activity prevents full MoDCs phenotypic maturation in response to DNCB and NiSO₄.

Inhibition of CK2 activity modulates cytokine production by MoDCs

We quantified cytokine production in MoDC supernatants by using an MSD multiplex assay. Cytokines were measured 24 h after NiSO₄ or DNCB stimulation of MoDCs, pretreated or not with CX-4945. Both NiSO₄ and DNCB stimulation induced the production of the proinflammatory cytokines IL-6 and TNF- α and the chemokines CCL-4 and CXCL-8 in MoDCs. Only NiSO₄ induced a relevant production of IL-1 β and -23 (Fig. 4A and B). In response to NiSO₄, inhibition of CK2 activity had no effect on CXCL-8 production, whereas production of IL-1 β and -23 was up-regulated compared to that in NiSO₄-stimulated MoDCs (Fig. 4A). Moreover, CX-4945 pretreatment led to a decrease in IL-6, TNF- α , and CCL-4 production in NiSO₄-stimulated MoDCs. In response to DNCB, CXCL-8 was down-regulated in MoDCs pretreated with CX-4945 (Fig. 4B). However, TNF- α production was increased in the absence of CK2 activity in DNCB-stimulated MoDCs.

Regarding IL-12p70, the absence of CK2 tended to decrease IL-12p70 production slightly, in response to NiSO₄. Because IL-12p70 is made-up of IL-12p40 and IL-12p35 subunits and IL-23 comprises IL-23p19 and IL-12p40, we measured the different subunits by RT-qPCR: *il12p35*, *il12p40*, and *il23p19*. In response to NiSO₄, mRNA expression of the 3 subunits mentioned above was up-regulated (Fig. 4C). In the absence of CK2 activity, mRNA expression of *il12p35* tended to be decreased, whereas *il12p40* and *il23p19* mRNA expression, subunits of IL-23, were significantly increased.

Taken together, these results are evidence that CK2 plays a role in the production of several proinflammatory cytokines by MoDCs in response to NiSO₄ and DNCB. *il12p35* is controlled positively, whereas *il12p40* and *il23p19* are negatively controlled by CK2 via a transcriptional mechanism.

Inhibition of CK2 activity impairs the capacity of DNCB- and NiSO₄-treated MoDCs to activate CD4⁺ T-lymphocyte proliferation

Because we showed that CK2 is involved in MoDCs' acquisition of a mature phenotype and production of cytokines, we then studied the capacity of CX-4945-pretreated MoDCs to activate CD4⁺ T lymphocytes. Although NiSO₄ and DNCB are known to trigger contact hypersensitivity, mainly through CD8⁺ cytotoxic effector T lymphocytes in mice [3], the question was the importance of CK2 activity in the functional capacity of human DCs to activate CD4⁺ T lymphocytes, a key step in initiating a full immune response [26].

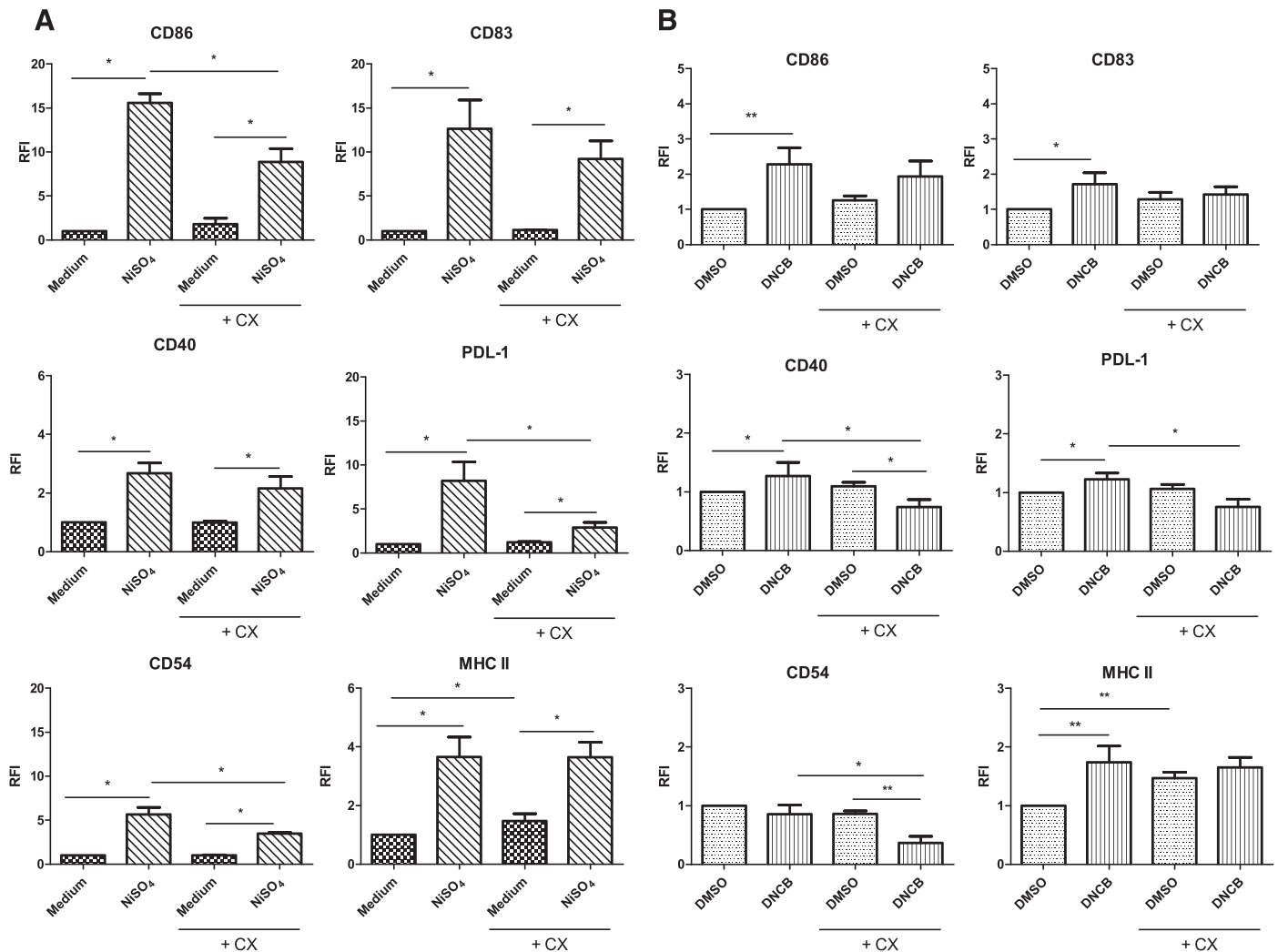


Figure 3. Inhibition of CK2 activity down-regulates the expression of key surface markers of MoDC maturation. Immature MoDCs were pretreated for 1 h with DMSO 0.1% or CX-4945 (CX, 5 μ M) and then stimulated for 24 h with NiSO₄ 500 μ M (A) or DNCB 10 μ M (B). After 24 h, the expression of cell surface markers was quantified by flow cytometry. Data represent the results of 5 independent experiments and are expressed as the fold increase over control medium (for NiSO₄) or DMSO (for DNCB). * P < 0.05; ** P < 0.01 (Mann-Whitney test).

NiSO₄- or DNCB-stimulated MoDCs, pretreated or not with CX-4945, were cocultured with allogeneic CD4⁺ T lymphocytes loaded with CFSE. Proliferation was evaluated on d 5 as a percentage of CFSE^{low} CD4⁺ T cells. In the absence of DC treatment with NiSO₄ or DNCB, <30% of CD4⁺ T cells were CFSE^{low}. Pretreatment of MoDCs with CX-4945 had no effect on CD4⁺ T cell proliferation in the absence of NiSO₄ or DNCB stimulation (Fig. 5A and 5B). NiSO₄- or DNCB-stimulated MoDCs augmented the proliferation of CD4⁺ T lymphocytes with $51 \pm 5\%$ and $41 \pm 3\%$ of CFSE^{low} cells, respectively. The proliferation of CD4⁺ T lymphocytes cocultured with MoDCs pretreated with CX-4945 and then stimulated with NiSO₄ or DNCB was significantly decreased ($36 \pm 5\%$ and $23 \pm 2\%$ of CFSE^{low} cells, respectively; Fig. 5A) without affecting the number of cell cycles of the proliferating cells (Fig. 5C). However, CX-4945 pretreatment led to a decrease in cell count in each generation, particularly in generations 5 and 6 with NiSO₄ and in 4 and 5 with DNCB. The collective findings reveal that CK2

activity is involved in the capacity of NiSO₄- or DNCB-stimulated MoDCs to augment CD4⁺ T cell proliferation. The data suggest that CK2 plays a role in the proliferation of T lymphocytes through DCs.

Inhibition of CK2 affects CD4⁺ T-cell polarization induced by DNCB- and NiSO₄-activated MoDCs

CK2 appears to have an important role in MoDCs' ability to activate CD4⁺ T-lymphocyte proliferation and to modulate key cytokine production known to be involved in T-cell polarization. We thus addressed its role in T-cell polarization. MoDCs were incubated for 24 h, with or without DNCB or NiSO₄, in the presence or absence of CX-4945, and then cocultured with allogeneic CD4⁺ T lymphocytes. Cytokine production of activated T lymphocytes was evaluated 5 d later by quantifying cytokines in cell culture supernatants with an MSD multiplex assay (Fig. 6). Because high variability was observed between donors, results are expressed as fold increase compared to control DMSO or

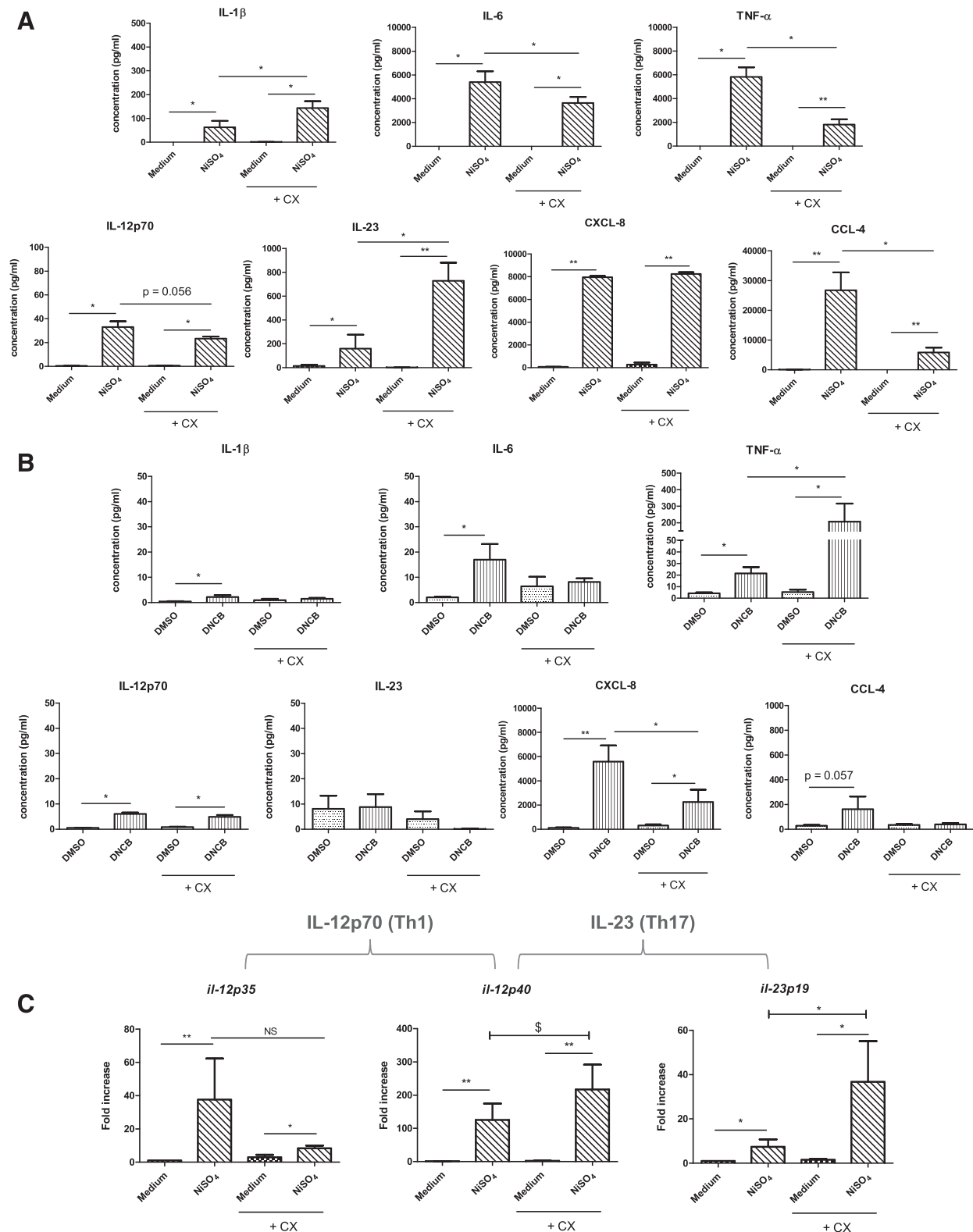


Figure 4. Inhibition of CK2 activity modulates cytokine production by MoDCs. Immature MoDCs were pretreated for 1 h with DMSO 0.1% or CX-4945 (CX, 5 μ M) and then stimulated for 24 h with NiSO₄ 500 μ M (A) or DNCB 10 μ M (B). Levels of IL-1 β , CXCL-8, IL-6, TNF- α , CCL-4, IL-12p70, and IL-23 were quantified in MoDCs supernatants with a Multi-Scale Discovery Multiplex assay. Immature MoDCs were pretreated for 1 h with DMSO 0.1% or CX-4945 (CX, 5 μ M) and then stimulated for 6 h with NiSO₄ 500 μ M. *il-12p35*, *il-12p40*, and *il-23p19* mRNAs were quantified by real-time RT-PCR (C). Data represent the results of 5 independent experiments and are expressed as means $\pm$ SEM (A and B) or as fold increase normalized to control (C). * P < 0.05; ** P < 0.01 (Mann-Whitney test).

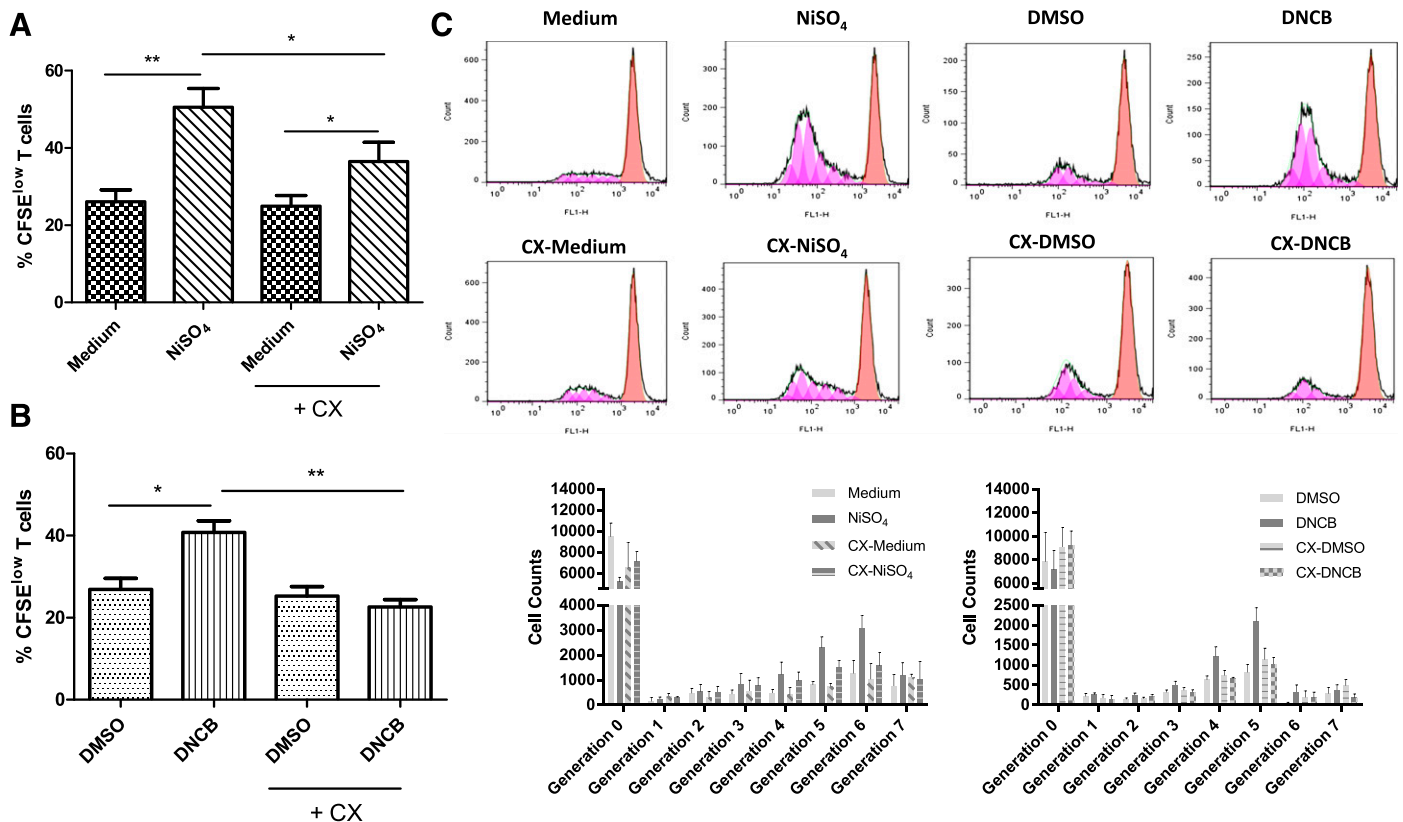


Figure 5. Inhibition of CK2 activity impairs DNCB but not NiSO₄ MoDC-induced CD4⁺ T-lymphocyte proliferation. CFSE-loaded CD4⁺ T lymphocytes were cultured with allogeneic MoDCs pretreated with (A) NiSO₄ 500 μ M or (B) DNCB 10 μ M (ratio 1 T cell: 10 DCs) in the presence or absence of CX-4945 (CX, 5 μ M). On day 6 the percentage of CFSE^{low} cells was determined by flow cytometry. Results are expressed as the percentage of CFSE⁺ cells, corresponding to T-cell proliferation or as cell counts per generation (C). The results are representative of 8 independent experiments. * $P < 0.05$; ** $P < 0.01$ (Mann-Whitney test).

medium. As expected, NiSO₄ or DNCB-stimulated MoDCs augmented the secretion of IFN- γ by T cells, whereas IL-17 was produced only in the presence of NiSO₄-stimulated DCs. In the absence of CK2 activity in DCs, secretion of IFN- γ by T cells was down-regulated in response to MoDCs treated with either chemical (Fig. 6A and B). However, IL-17 production was maintained in the absence of CK2 activity, in response to MoDCs treated with NiSO₄ (Fig. 6A). These results suggest that CK2 in DCs controls Th1 polarization by controlling T-cell IFN- γ secretion in response to both NiSO₄ and DNCB (Fig. 6A and B).

Analysis of naïve allogeneic CD4⁺ T-cell supernatants, cocultured for 5 d with MoDCs pretreated or not with CX-4945 and stimulated with NiSO₄, showed that CK2 controlled IFN- γ secretion without affecting IL-17 production in naïve CD4⁺ T cells. As previously shown by Turbica et al. [27] in naïve CD4⁺ T cells, NiSO₄ stimulation induced IL-5 and -13 production contrary to total CD4⁺ T cells. Solely in the absence of CK2 activity in untreated MoDCs, IL-5 and -13 production was enabled.

These results suggest that CK2 activity could negatively regulate IL-5 and -13 expression in response to unstimulated MoDCs (basal state).

Taken together, these data indicate that CK2 is involved in T-cell polarization, both in the basal state and in stimulated

conditions. CK2 seemed to particularly control Th1 response by modulating IFN- γ production in response to NiSO₄ and DNCB.

Inhibition of CK2 activity modulates key transcription factors involved in DC and in T-cell polarization

The initiation of (Th1/Th17)/Th2 response was dependent on the expression of members of the IRF family in DCs and other transcription factors, such as T-Bet, known to be involved in Th1 response, ROR γ T for Th17 polarization, and GATA-3, known to be involved in Th2 polarization.

First, we analyzed by RT-qPCR the expression in MoDCs of the main IRFs described in T-cell polarization: *irf-1*, -4, and -8. NiSO₄ stimulation induced the up-regulation of expression of *irf-1*, -8, and -4 mRNA (Fig. 7A), whereas CX-4945 pretreatment of MoDCs before NiSO₄-stimulation led to a strong down-regulation of all the tested IRF mRNA expression. DNCB stimulation induced mRNA expression of *irf-1*, down-regulated mRNA expression of *irf-4*, and did not modulate *irf-8* mRNA (Fig. 7B). Loss of CK2 activity with CX-4945 pretreatment before DNCB stimulation led to a significant decrease in *irf-1* mRNA expression. *Ir-4* mRNA expression in MoDCs was significantly increased in the absence of CK2 activity without chemical stimulation (Fig. 7A and B). DNCB and NiSO₄ induced the expressions of *irf* mRNA in a different manner. However, the

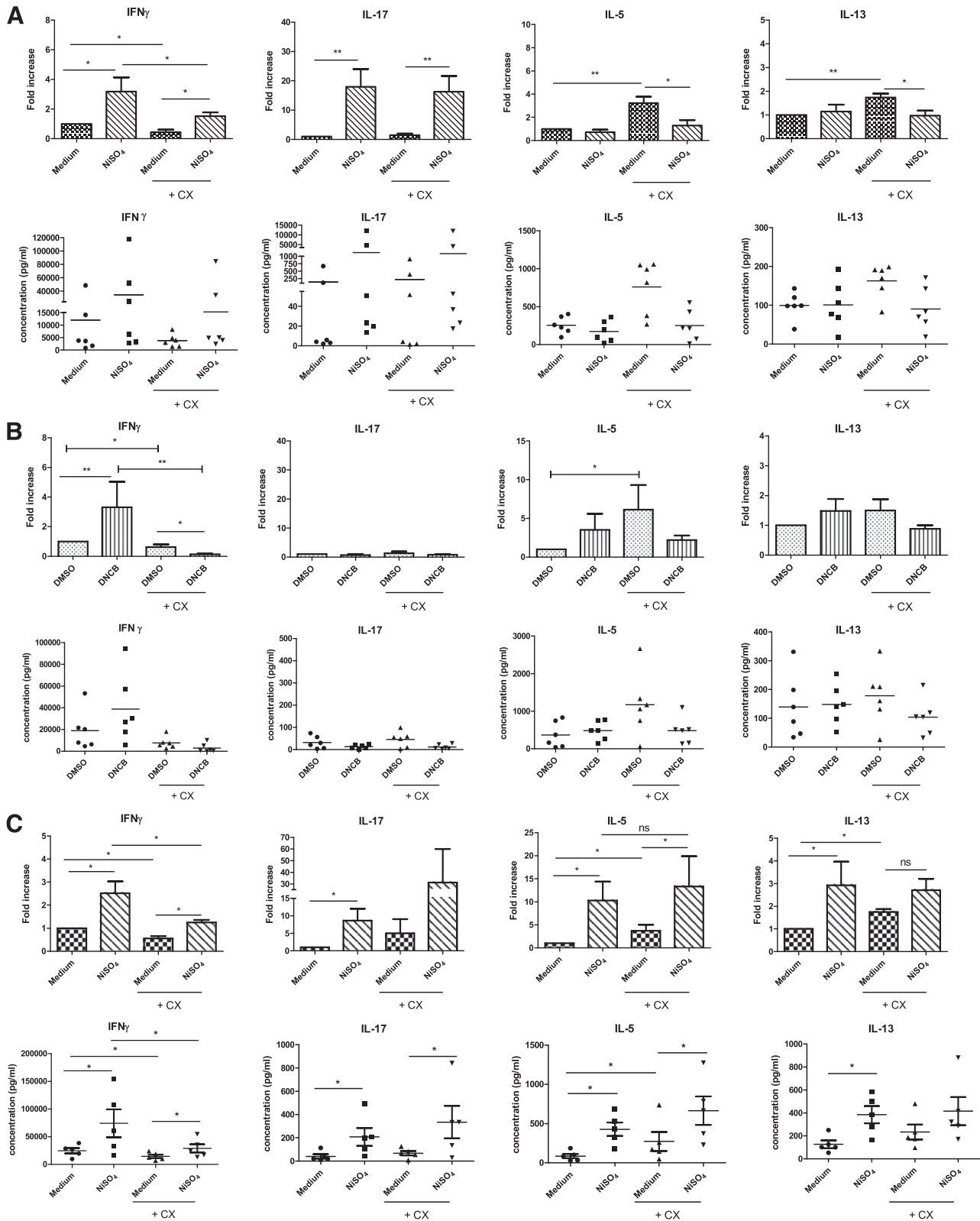


Figure 6. Inhibition of CK2 activity modulates DNCB and NiSO $_4$ MoDCs-induced CD4 $^+$ T lymphocyte polarization. Total CD4 $^+$ T lymphocytes were coincubated with MoDCs, both immature and NiSO $_4$ - (A) and DNCB- (B) treated, and naïve CD4 $^+$ T lymphocytes (C) were coincubated with NiSO $_4$ -stimulated MoDCs in the absence or presence of CX-4945 (5 μ M). On day 5, cytokines were quantified in the coculture supernatants using a Multi-Scale Discovery Multiplex assay. Results are expressed as fold increase compared to control medium or DMSO; or are shown as dot plots. The results represent 6 (A and B) or 5 (C) independent experiments. * P < 0.05; ** P < 0.01 (Mann-Whitney test).

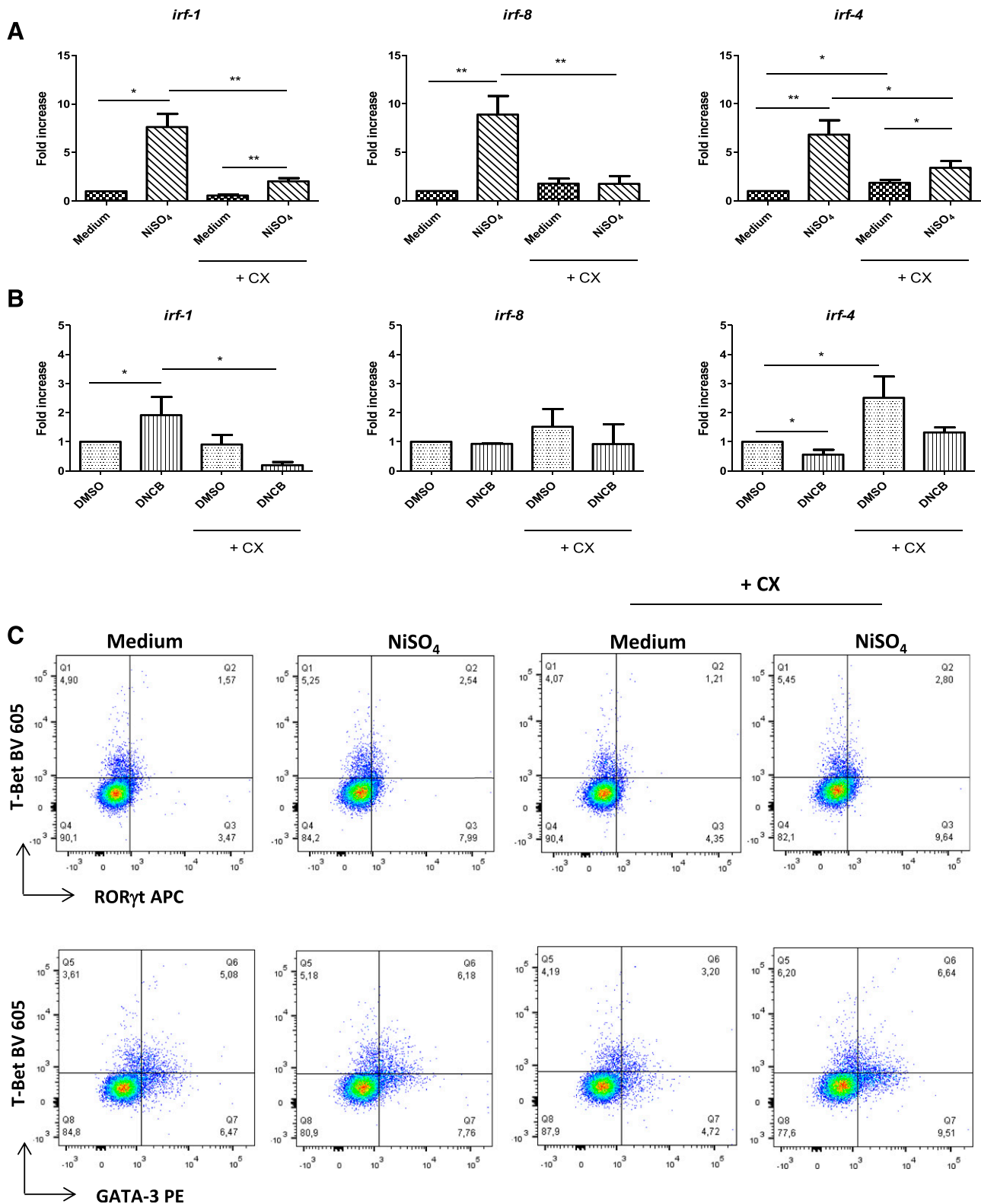


Figure 7. Inhibition of CK2 activity modulates mRNA expression of IRFs in MoDCs. Immature MoDCs were pretreated for 1 h with DMSO 0.1% or CX-4945 (CX, 5 μM) and then stimulated for 6 h with (A) NiSO_4 500 μM or (B) DNCB 10 μM . IRF-1, -8, and -4 mRNAs were quantified by real-time RT-PCR. Naïve CD4^+ T lymphocytes were coincubated with NiSO_4 -stimulated MoDCs (C) in the absence or presence of CX-4945 (5 μM). On day 5, cells were restimulated with anti-CD3/CD2/CD28 beads for 48 h and stained with CD4-Alexa Fluor 700, CD3-FITC, Ror γ T-APC, GATA3-PE, and T-Bet Brilliant Violet 605. Living cells (ZombieAqua negative cells) were analyzed by flow cytometry. Data represent the results of 5 independent experiments and are normalized to the control. * $P < 0.05$; ** $P < 0.01$ (Mann-Whitney test).

3 IRFs (*irf-1*, *-4*, and *-8*) induced in response to NiSO₄ and *irf-1* induced by DNCB stimulation was regulated by CK2.

The analysis of other transcription factors using intracellular staining allowed us to show that NiSO₄-stimulated MoDCs induced slight T-Bet (from 6.5 to 7.8%, and from 8.7 to 11.4%), GATA-3 (from 11.5 to 13.9%), and ROR γ T (from 5 to 10.5%) expression (Fig. 7C). CX-4945 pretreatment of NiSO₄-stimulated MoDCs did not modulate T-Bet expression, whereas ROR γ T and GATA-3 expression was increased (from 10.5 to 12.5% and from 13.9 to 16.2%, respectively). GATA-3 was decreased in CX-4945-pretreated control cells, suggesting that the up-regulation of Th2 cytokines (IL-5 and -13) could be measured independent of GATA-3, probably because the T-cell population was not highly polarized.

DISCUSSION

Allergic contact dermatitis is a T-cell inflammatory disease wherein DCs play a crucial role. Chemical sensitizers like NiSO₄ and DNCB have been demonstrated to induce the expression of markers related to DC maturation and function and the production of proinflammatory cytokines. Furthermore, these chemicals induced the phosphorylation of MAPK family proteins and the activation of the NF- κ B pathway, mimicking the effects of danger signals and leading to the acquisition of a mature phenotype by DCs [7–9, 11–13].

Using a peptide array approach, we identified a novel kinase, protein kinase CK2 (formerly casein kinase 2), that plays a role in the acquisition of a mature phenotype in DCs in response to DNCB and NiSO₄, well-known contact sensitizers. CK2 has been described to participate in the activation of several major signaling pathways as Ras-Raf-MEK-ERK, p38 MAPK, PKC, and PI3 kinase-Akt and to phosphorylate different MAPK effectors [28].

In this work, we studied the role of CK2 in DC activation in response to contact sensitizers. Our data are the first to demonstrate such a role for CK2 in DCs. The inhibition of CK2 prevented NiSO₄- or DNCB-induced DC maturation and impaired T-cell proliferation by these well-known contact allergen inducers of DC maturation.

In response to contact allergens, *in vitro*, DCs preferentially secrete cytokines that polarize the T-cell response toward Tc1/Th1 and Tc17/Th17 effector T cells [29]. In ACD caused by nickel, there is a massive cellular infiltration dominated by CD4⁺ T cells producing IL-17 and IFN- γ in nickel-challenged skin [30]. Pickard et al. [26] also showed that DNCB leads to Th1 and Tc1 responses in humans exposed to this chemical. In our hands, the absence of CK2 activity led to an alteration of the cytokine profile secreted by T lymphocytes: secreting less IFN- γ in response to both chemicals, whereas IL-17 production was maintained in response to NiSO₄. In the absence of CK2 activity in NiSO₄-stimulated MoDC, the up-regulation of IL-23 and -1 β could promote Th17 polarization vs. Th1/Th17 [31]. Furthermore, allogeneic T CD4⁺ lymphocytes proliferation was affected. These data support the notion that CK2 activity in DCs drives T-cell polarization by preferentially inducing a Th1 response to contact sensitizers without modulating Th17 polarization.

The IRF family can directly alter the transcription of cytokine genes by Th cells, and the IRF family controls many aspects of DC biology [32]. IRFs govern the development of various DC subsets and promote and modulate DC maturation [33]. The main IRF transcription factors involved in T-cell polarization are IRF-1, -4, and -8. IRF-1 and -8 are involved in IL-12 synthesis and drive Th1/Th17 response [32, 34], whereas IRF-4 controls Th2 response [35, 36]. In the present work, *irf-1* mRNA was induced in response to both chemicals, whereas *irf-4* and *irf-8* mRNAs were induced only in response to NiSO₄. We found that DCs stimulated with NiSO₄ are IRF1⁺IRF8⁺IRF4⁺PDL1^{high}CD54^{high} DCs, allowing a Th1/Th17 response, and DNCB-stimulated DCs were IRF1⁺PDL1^{low}CD54^{low}, allowing a Th1 response. Inhibition of CK2 activity could convert these DCs into IRF1⁺IRF8⁺IRF4⁺PDL1^{low}CD54^{low} DCs in response to NiSO₄ and into IRF1⁺PDL1⁺CD54⁺ DCs in response to DNCB. Our results showed that CK2 regulated *irf-8* positively, whereas it negatively regulated *il23p19* and *il12p40* mRNA. IL-23, mainly released by DCs, was an essential component for the maintenance but not the induction of Th17 cells [37]. The induction of IL-23 seems to be independent of IRF-8 and suggests that other transcription factors are regulated by CK2, such as NFAT and IRF-4 [38]. Accordingly, the negative regulation of IRF1 and -8 in MoDC in absence of CK2 activity is in accordance with the maintenance of Th17 polarization in response to NiSO₄. These results correlated very well with the higher percentage of ROR γ ⁺ cells in response to NiSO₄ pretreated with CX-4945 compared to NiSO₄ alone. In response to DNCB, the low IRF-1 expression could explain the low production of IL-12, a key cytokine for Th1 polarization.

The absence of CK2 in unstimulated DCs leads to a spontaneous Th2 polarization. Our results suggest that CK2 activity in MoDC could control the DC phenotype prone to Th2 polarization, as shown by Ulges et al. [39]. Their study demonstrated that CK2 allows regulatory T cells to suppress excessive Th2 response *in vivo* [39]. In our study, these cells were T-Bet⁺GATA3⁺ROR γ ⁺IL-5⁺IL-13⁺IL-17⁺IFN- γ ⁺. T-Bet and GATA3 are frequently coexpressed in different lineages, and the balanced interplay between them dictates the resultant cell fate [40]. It has been shown that T-Bet undergoes several post-translational protein modifications, such as phosphorylation on tyrosine, serine, and threonine residues, affecting Th cell differentiation [41]. The absence of CK2 activity in MoDC may alter indirectly some signaling pathways involved in T-cell activation, particularly in T-Bet phosphorylation. However, the signal provided by contact allergen stimulation inhibits the Th2 response induced in the absence of CK2 activity.

Our study also shows that CK2 negatively controls *irf-4* transcription in unstimulated MoDCs. Because IRF-4 has been shown to be a key regulator of DC-Th2 development [42], we could propose that *irf-4* mRNA induction in MoDCs explains the spontaneous Th2 polarization. Neither PDL-2 nor OX40L, markers described as Th2 inducers, are induced in the absence of CK2 activity in unstimulated MoDCs (data not shown). Ulges et al. [39] have demonstrated that the genetic ablation of the β -subunit of CK2, specifically in Tregs, results in the maturation of IRF4⁺PDL-2⁺ DCs, required for the development of Th2 response *in vivo*. In our study, NiSO₄ inducing IRF4⁺PDL1⁺ DCs were also required for naive Th2 polarization.

In conclusion, this work contributes to a better understanding of the role of CK2 in DC–T cell crosstalk, as recently described [39, 43, 44], and highlights CK2 as a key kinase in chemical sensitizer-induced DC phenotype modulation, cytokine production, and CD4⁺ T-lymphocyte polarization. CK2 controls Th1 response through IFN- γ secretion by T cells and maintains Th17 polarization through IL-1 β and -23 production by DCs, independent of IRF8. As a transcription modulator, CK2 triggers an efficient immune response because this kinase modulates mRNA expression of several cytokines such as IL-23 and transcription factors involved in T-cell response. Our results also suggest that CK2 is a key kinase in the control of the Th2 response, probably through IRF-4. Our study revealed a novel function of CK2 in DC in response to contact sensitizers.

AUTHORSHIP

M.d.B. designed the study, performed experiments, analyzed data, and wrote the manuscript. Y.G., Z. E. A., Ph.R., and M.-H.D. performed experiments. C.C. and O.F. participated in CK2 data analysis. S.C.M. participated in writing the manuscript. M.P. analyzed data and wrote the manuscript. S.K.-R. designed the study, performed experiments, analyzed data, and wrote the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

This work was supported by grants from the Domaine d'Intérêt Majeur (DIM) de l'Ile de France "Santé et environnement" (to M.d.B.) and the Region Ile-de-France and ANR 11-CESA-015-01–Allergochem, and ANR 2007 SEST 002 01–AllergoChem. The authors thank the UMS Institut Paris Saclay d'Innovation Thérapeutique (IPSIT), Plateforme Trans-Prot, Université of Paris-Sud, Université Paris-Saclay, Plateforme d'immuno-monitorage PLAIMMO, Institut Paris-Saclay d'Innovation Thérapeutique, and Marie-Laure Aknin and Claudine Deloménie for technical assistance.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

1. Peiser, M., Tralau, T., Heidler, J., Api, A. M., Arts, J. H., Basketter, D. A., English, J., Diepgen, T. L., Fuhlbrigge, R. C., Gaspari, A. A., Johansen, J. D., Karlberg, A. T., Kimber, I., Lepoittevin, J. P., Liebsch, M., Maibach, H. I., Martin, S. F., Merk, H. F., Platzeck, T., Rustemeyer, T., Schnuch, A., Vandebriel, R. J., White, I. R., Luch, A. (2012) Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects: current knowledge assembled at an international workshop at BfR, Germany. *Cell. Mol. Life Sci.* **69**, 763–781.
2. Kaplan, D. H., Igyártó, B. Z., Gaspari, A. A. (2012) Early immune events in the induction of allergic contact dermatitis. *Nat. Rev. Immunol.* **12**, 114–124.
3. Vocanson, M., Hennino, A., Rozières, A., Poyet, G., Nicolas, J. F. (2009) Effector and regulatory mechanisms in allergic contact dermatitis. *Allergy* **64**, 1699–1714.
4. Freudenberg, M. A., P. R. Esser, T. Jakob, C. Galanos, S. F. Martin. 2009. Innate and adaptive immune responses in contact dermatitis: analogy with infections. *G. Ital. Dermatol. Venereol.* **144**, 173–185.
5. Warshaw, E. M., Belsito, D. V., Taylor, J. S., Sasseville, D., DeKoven, J. G., Zirwas, M. J., Fransway, A. F., Mathias, C. G., Zug, K. A., DeLeo, V. A., Fowler, J. F., Jr., Marks, J. G., Pratt, M. D., Storrs, F. J., Maibach, H. I. 2013. North American Contact Dermatitis Group patch test results: 2009 to 2010. *Dermatitis* **24**, 50–59.
6. Friedmann, P. S., Moss, C., Shuster, S., Simpson, J. M. (1983) Quantitation of sensitization and responsiveness to dinitrochlorobenzene in normal subjects. *Br. J. Dermatol.* **109**(Suppl 25), 86–88.
7. Boisleve, F., Kerdine-Römer, S., Rougier-Larzat, N., Pallardy, M. (2004) Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-alpha and MAPK. *J. Invest. Dermatol.* **123**, 494–502.
8. Ade, N., Antonios, D., Kerdine-Römer, S., Boisleve, F., Rousset, F., Pallardy, M. (2007) NF-kappaB plays a major role in the maturation of human dendritic cells induced by NiSO(4) but not by DNCB. *Toxicol. Sci.* **99**, 488–501.
9. Aiba, S., Manome, H., Nakagawa, S., Mollah, Z. U. A., Mizuashi, M., Ohtani, T., Yoshino, Y., Tagami, H. (2003) p38 Mitogen-activated protein kinase and extracellular signal-regulated kinases play distinct roles in the activation of dendritic cells by two representative haptens, NiCl2 and 2,4-dinitrochlorobenzene. *J. Invest. Dermatol.* **120**, 390–399.
10. Toebak, M. J., Pohlmann, P. R., Sampat-Sardjoeppersad, S. C., von Blomberg, B. M. E., Bruynzeel, D. P., Scheper, R. J., Rustemeyer, T., Gibbs, S. (2006) CXCL8 secretion by dendritic cells predicts contact allergens from irritants. *Toxicol. In Vitro* **20**, 117–124.
11. Arrighi, J. F., Rebsamen, M., Rousset, F., Kindler, V., Hauser, C. (2001) A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF-alpha, and contact sensitizers. *J. Immunol.* **166**, 3837–3845.
12. Boisleve, F., Kerdine-Römer, S., Pallardy, M. (2005) Implication of the MAPK pathways in the maturation of human dendritic cells induced by nickel and TNF-alpha. *Toxicology* **206**, 233–244.
13. Antonios, D., Rousseau, P., Larangé, A., Kerdine-Römer, S., Pallardy, M. (2010) Mechanisms of IL-12 synthesis by human dendritic cells treated with the chemical sensitizer NiSO4. *J. Immunol.* **185**, 89–98.
14. Neves, B. M., Gonçalves, M., Figueiredo, A., Duarte, C. B., Lopes, M. C., Cruz, M. T. (2011) Signal transduction profile of chemical sensitizers in dendritic cells: an endpoint to be included in a cell-based in vitro alternative approach to hazard identification? *Toxicol. Appl. Pharmacol.* **250**, 87–95.
15. Singh, N. N., Ramji, D. P. (2008) Protein kinase CK2, an important regulator of the inflammatory response? *J. Mol. Med.* **86**, 887–897.
16. Pierre, F., Chua, P. C., O'Brien, S. E., Siddiqui-Jain, A., Bourbon, P., Haddach, M., Michaux, J., Nagasawa, J., Schwaebé, M. K., Stefan, E., Viallettes, A., Whitten, J. P., Chen, T. K., Darjania, L., Stansfield, R., Bliesath, J., Drygin, D., Ho, C., Omori, M., Proffitt, C., Streiner, N., Rice, W. G., Ryckman, D. M., Anderes, K. (2011) Pre-clinical characterization of CX-4945, a potent and selective small molecule inhibitor of CK2 for the treatment of cancer. *Mol. Cell. Biochem.* **356**, 37–43.
17. Battistutta, R., Cozza, G., Pierre, F., Papinutto, E., Lolli, G., Sarno, S., O'Brien, S. E., Siddiqui-Jain, A., Haddach, M., Anderes, K., Ryckman, D. M., Meggio, F., Pinna, L. A. (2011) Unprecedented selectivity and structural determinants of a new class of protein kinase CK2 inhibitors in clinical trials for the treatment of cancer. *Biochemistry* **50**, 8478–8488.
18. Filhol, O., Cochet, C., Wedegaertner, P., Gill, G. N., Chambaz, E. M. (1991) Coexpression of both alpha and beta subunits is required for assembly of regulated casein kinase II. *Biochemistry* **30**, 11133–11140.
19. Ma, C., Muranyi, M., Chu, C. H., Zhang, J., Chu, W.-M. (2013) Involvement of DNA-PKcs in the IL-6 and IL-12 response to CpG-ODN is mediated by its interaction with TRAF6 in dendritic cells. *PLoS One* **8**, e58072.
20. Cejas, P. J., Carlson, L. M., Zhang, J., Padmanabhan, S., Kolonias, D., Lindner, I., Haley, S., Boise, L. H., Lee, K. P. (2005) Protein kinase C betaII plays an essential role in dendritic cell differentiation and autoregulates its own expression. *J. Biol. Chem.* **280**, 28412–28423.
21. Rescigno, M., Martino, M., Sutherland, C. L., Gold, M. R., Ricciardi-Castagnoli, P. (1998) Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med.* **188**, 2175–2180.
22. Koga, Y., Matsuzaki, A., Suminoe, A., Hattori, H., Hara, T. (2008) Expression of cytokine-associated genes in dendritic cells (DCs): comparison between adult peripheral blood- and umbilical cord blood-derived DCs by cDNA microarray. *Immunol. Lett.* **116**, 55–63.
23. Yanagawa, Y., Iijima, N., Iwabuchi, K., Onoe, K. (2002) Activation of extracellular signal-related kinase by TNF-alpha controls the maturation and function of murine dendritic cells. *J. Leukoc. Biol.* **71**, 125–132.
24. Pierre, F., Chua, P. C., O'Brien, S. E., Siddiqui-Jain, A., Bourbon, P., Haddach, M., Michaux, J., Nagasawa, J., Schwaebé, M. K., Stefan, E., Viallettes, A., Whitten, J. P., Chen, T. K., Darjania, L., Stansfield, R., Anderes, K., Bliesath, J., Drygin, D., Ho, C., Omori, M., Proffitt, C., Streiner, N., Trent, K., Rice, W. G., Ryckman, D. M. (2011) Discovery and SAR of 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid (CX-4945), the first clinical stage inhibitor of protein kinase CK2 for the treatment of cancer. *J. Med. Chem.* **54**, 635–654.
25. Antonios, D., Ade, N., Kerdine-Römer, S., Assaf-Vandecasteele, H., Larangé, A., Azouri, H., Pallardy, M. (2009) Metallic haptens induce differential phenotype of human dendritic cells through activation of mitogen-activated protein kinase and NF-kappaB pathways. *Toxicol. In Vitro* **23**, 227–234.

26. Pickard, C., Smith, A. M., Cooper, H., Strickland, I., Jackson, J., Healy, E., Friedmann, P. S. (2007) Investigation of mechanisms underlying the T-cell response to the hapten 2,4-dinitrochlorobenzene. *J. Invest. Dermatol.* **127**, 630–637.
27. Turbica, I., Gallais, Y., Gueguen, C., Tharinger, H., Al Sabbagh, C., Gorges, R., Gary-Gouy, H., Kerdine-Römer, S., Pallardy, M., Mascarell, L., Gleizes, A., Chollet-Martin, S. (2015) Ectosomes from neutrophil-like cells down-regulate nickel-induced dendritic cell maturation and promote Th2 polarization. *J. Leukoc. Biol.* **97**, 737–749.
28. Meggio, F., Pinna, L. A. (2003) One-thousand-and-one substrates of protein kinase CK2? *FASEB J.* **17**, 349–368.
29. Martin, S. F. (2015) New concepts in cutaneous allergy. *Contact Dermat.* **72**, 2–10.
30. Dyring-Andersen, B., Skov, L., Løvendorf, M. B., Bzorek, M., Søndergaard, K., Lauritsen, J. P. H., Dabelsteen, S., Geisler, C., Bonefeld, C. M. (2013) CD4(+) T cells producing interleukin (IL)-17, IL-22 and interferon- γ are major effector T cells in nickel allergy. *Contact Dermat.* **68**, 339–347.
31. McFadden, J. P., Puangpet, P., Basketter, D. A., Dearman, R. J., Kimber, I. (2013) Why does allergic contact dermatitis exist? *Br. J. Dermatol.* **168**, 692–699.
32. Lohoff, M., Mak, T. W. (2005) Roles of interferon-regulatory factors in T-helper-cell differentiation. *Nat. Rev. Immunol.* **5**, 125–135.
33. Gabriele, L., Ozato, K. (2007) The role of the interferon regulatory factor (IRF) family in dendritic cell development and function. *Cytokine Growth Factor Rev.* **18**, 503–510.
34. Yoshida, Y., Yoshimi, R., Yoshii, H., Kim, D., Dey, A., Xiong, H., Munasinghe, J., Yazawa, I., O'Donovan, M. J., Maximova, O. A., Sharma, S., Zhu, J., Wang, H., Morse III, H. C., Ozato, K. (2014) The transcription factor IRF8 activates integrin-mediated TGF- β signaling and promotes neuroinflammation. *Immunity* **40**, 187–198.
35. Gao, Y., Nish, S. A., Jiang, R., Hou, L., Licona-Limón, P., Weinstein, J. S., Zhao, H., Medzhitov, R. (2013) Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* **39**, 722–732.
36. Rengarajan, J., Mowen, K. A., McBride, K. D., Smith, E. D., Singh, H., Glimcher, L. H. (2002) Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. *J. Exp. Med.* **195**, 1003–1012.
37. Hermann-Kleiter, N., Baier, G. (2010) NFAT pulls the strings during CD4+ T helper cell effector functions. *Blood* **115**, 2989–2997.
38. Stritesky, G. L., Yeh, N., Kaplan, M. H. (2008) IL-23 promotes maintenance but not commitment to the Th17 lineage. *J. Immunol.* **181**, 5948–5955.
39. Ulges, A., Klein, M., Reuter, S., Gerlitzki, B., Hoffmann, M., Grebe, N., Staudt, V., Stergiou, N., Bohn, T., Brühl, T.-J., Muth, S., Yurugi, H., Rajalingam, K., Bellinghausen, I., Tuettenberg, A., Hahn, S., Reissig, S., Haben, I., Zipp, F., Waisman, A., Probst, H.-C., Beilhack, A., Buchou, T., Filhol-Cochet, O., Boldyreff, B., Breloer, M., Jonuleit, H., Schild, H., Schmitt, E., Bopp, T. (2015) Protein kinase CK2 enables regulatory T cells to suppress excessive TH2 responses in vivo. *Nat. Immunol.* **16**, 267–275.
40. Evans, C. M., Jenner, R. G. (2013) Transcription factor interplay in T helper cell differentiation. *Brief. Funct. Genomics* **12**, 499–511.
41. Oh, S., Hwang, E. S. (2014) The role of protein modifications of T-bet in cytokine production and differentiation of T helper cells. *J. Immunol. Res.* **2014**, 589672.
42. Williams, J. W., Tjota, M. Y., Clay, B. S., Vander Lugt, B., Bandukwala, H. S., Hrusch, C. L., Decker, D. C., Blaine, K. M., Fixsen, B. R., Singh, H., Sciammas, R., Sperling, A. I. (2013) Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat. Commun.* **4**, 2990.
43. Sawant, D. V., Dent, A. L. (2015) It takes CK2 to suppress TH2. *Nat. Immunol.* **16**, 224–225.
44. Bird, L. (2015) Regulatory T cells: CK2: keeping TH2 cells in check. *Nat. Rev. Immunol.* **15**, 134.

KEY WORDS:

dendritic cell · protein kinase · Th1/Th2/Th17 cell · allergic contact dermatitis · cytokine

Protein kinase CK2 controls T-cell polarization through dendritic cell activation in response to contact sensitizers

Marie de Bourayne, Yann Gallais, Zeina El Ali, et al.

J Leukoc Biol 2017 101: 703-715 originally published online October 5, 2016
Access the most recent version at doi:[10.1189/jlb.3A0715-320RR](https://doi.org/10.1189/jlb.3A0715-320RR)

References This article cites 44 articles, 12 of which can be accessed free at:
<http://www.jleukbio.org/content/101/3/703.full.html#ref-list-1>

Subscriptions Information about subscribing to *Journal of Leukocyte Biology* is online at
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Permissions Submit copyright permission requests at:
http://www.jleukbio.org/site/misc/Librarians_Resource.xhtml

Email Alerts Receive free email alerts when new an article cites this article - sign up at
<http://www.jleukbio.org/cgi/alerts>
