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IL-27 production and regulation in human dendritic cells treated with the chemical sensitizer NiSO₄ .

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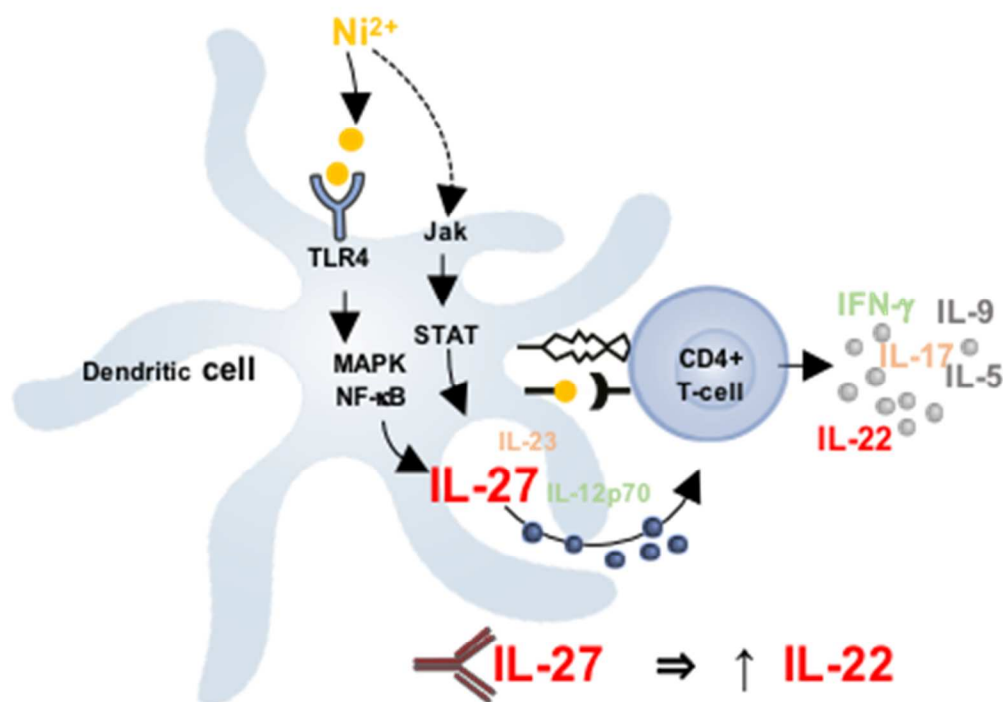
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Running Title: IL-27 regulation by nickel

Table of Contents Graphic



ABSTRACT

Allergic contact dermatitis (ACD) is a major cause of occupational skin disease and nickel is among the most prevalent contact allergens. Dendritic cells (DC) play an important role in ACD and in the type of the ensuing immune response through differential phenotypes and cytokine production. The interleukin (IL)-12 cytokine family is composed of heterodimeric cytokines sharing homology at the subunit, receptor, and signaling levels. We previously showed that nickel can upregulate the production of IL-12p40 and IL-23, both known to be pro-inflammatory. In this work, we aimed to extend our knowledge on nickel-regulation of the IL-12 cytokine family by focusing on IL-27, a recently identified immunomodulatory cytokine from this family. We showed that nickel induced the production of IL-27 in human monocyte-derived DC (MoDC) regulating IL-22 production by human CD4⁺ T cells. We also showed that nickel was able to induce the expression of the two subunits of IL-27: il-27p28 and ebi3. Furthermore, we demonstrated that the production of IL-27 was dependent on the TLR4, p38 MAPK, NF- κ B and Jak-STAT signaling. However, IL-27 subunits were differentially regulated by these pathways. Indeed, both subunits were positively regulated by the TLR4 and NF- κ B pathways but only il-27p28 was also dependent on p38 MAPK and Jak-STAT pathway. Our results contribute to a better understanding of nickel-induced ACD by focusing on the IL-12 cytokine family and elucidating the mechanism of IL-27 regulation in human dendritic cells.

1 INTRODUCTION

Allergic contact dermatitis (ACD) is a common inflammatory skin disease caused by an unnecessary immune response to topically applied allergens¹. According to a large epidemiological study, 27 % of the general population in 5 European countries suffer from ACD to at least one of the common contact allergens and nickel was among the most prevalent one². In fact, metal allergy is the most frequent type of contact allergy, with nickel allergy affecting 14.5 % of the European population². People are mostly exposed to metals by contact with jewelry, clothing ornamentation and coins. From an immunological point of view, ACD is the result of T-cell activation mediated by T cells recognizing low-molecular-weight chemicals or metals ions presented by antigen-presenting cells such as dendritic cells (DC)³. However, the presence of the contact sensitizer *per se* is usually insufficient to promote a T-cell response. A second signal, noted “danger signal”, is mandatory for effective DC activation and subsequent T-cell priming¹. Nickel is the perfect example of a “complete” contact sensitizer that follows this “two-signal” hypothesis. Alongside its ability to act as an antigen⁴, nickel ions can directly trigger activation of human Toll-like receptor 4 (TLR4) on DC⁵. As a consequence, intracellular signaling pathways, such as mitogen-activated protein kinase (MAPK), NF- κ B and interferon regulatory factor (IRF)-1 signaling pathways^{6–12}, are activated in DC leading to their maturation. Thereby, activated DC produce various cytokines and chemokines required for T-cell proliferation and polarization.

It is well known that the IL-12 heterodimeric cytokine family plays an important role in driving T-cell polarization and linking innate immunity with the development and the regulation of adaptive immunity¹³. This family, which includes IL-12p70, IL-23, IL-27 and IL-35, consist of an α -chain (p19, p28 or p35) and a β -chain (p40 or Ebi3). Despite chain-pairing similarities, the

IL-12 cytokine members have different biological activities. IL-12p70 and IL-23 are both considered to be pro-inflammatory and implicated in the development of Th1 and Th17 cells, whereas different immunomodulatory roles for IL-27 have been reported¹³. We have previously shown that NiSO₄ induced an early production of IL-23 by DC and a higher IL-23/IL-12p70 ratio, promoting Th17 cell development regulated by the TLR4 and Jak-STAT signaling pathways¹⁴. In this work, we aimed to expand our knowledge on nickel-modulation of the IL-12 family members by focusing on IL-27 regulation in human DC. Our results showed that nickel was able to induce the production of IL-27 in human monocyte-derived DC (MoDC), in a concentration dependent manner, and the expression of both IL-27 subunits: il-27p28 and ebi3. The inhibition of IL-27 produced by nickel-activated MoDC increased IL-22 production by allogeneic CD4⁺ T cells and did not affect T-cell proliferation. Moreover, we found that the TLR4, p38MAPK, NF-κB and Jak-STAT pathways played an important role in IL-27 production by nickel-treated MoDC. Although both subunits were positively regulated by TLR4 and NF-κB pathways, only the il-27p28 subunit was also regulated by the Jak-STAT and p38 MAPK pathways suggesting the implication of different signaling pathways in IL-27 subunits expression. Our results contribute to a better understanding of nickel-induced ACD by focusing on IL-12 cytokine family regulation and elucidating a novel role for IL-27.

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47 MATERIALS AND METHODS

48 Generation of human MoDC.

49 Buffy coats were collected from the Etablissement Français du Sang (EFS, Rennes, France) from
50 anonymous healthy donors after approval for experiments and written informed consent.
51 Peripheral blood mononuclear cells were purified from buffy coats by density centrifugation on a
52 Ficoll gradient (lymphocyte separation medium; PAA, Les Mureaux, France). The preparation of
53 MoDC was previously described ¹².

54 Chemical treatment of immature MoDC.

55 MoDC were treated or not with nickel sulfate (NiSO_4 ; 500 μM), nickel chloride (NiCl_2 ; 500
56 μM), and LPS (25 ng/ml) (Sigma-Aldrich, USA) for the indicated time. In some experiments,
57 MoDC were treated with IFN- γ (1000 U/ml, Abcys). To study the involvement of signaling
58 pathways in IL-27 production, MoDC were either pretreated for 1 h with anti-TLR4 or isotype
59 control (BioLegend, San Diego), for 30 min with SB203580 (20 μM), for 1 h with BAY 11-7085
60 (3 μM) or for 2 hrs with Jak Inhibitor I (0.5 μM) (Merck Chemicals, Darmstadt, Germany).
61 NiSO_4 , NiCl_2 and SB203580 were diluted in Ultrapure water (milli-Q®, Millipore corporation).
62 BAY 11-7085 and Jak inhibitor I were both diluted in dimethyl sulfoxide DMSO (<0.1%;
63 Sigma-Aldrich). Cell viability after treatment was never less than 75% as assessed by trypan blue
64 exclusion or propidium iodide.

65 RNA isolation and real-time RT-PCR analysis.

66 Total RNA was extracted after lysis of treated MoDC in RNA-PLUS reagent (MP Biomedicals,
67 USA). qRT-PCR was performed using the SYBR Green technology. Quantification was

performed with Bio-Rad Laboratories CFX Manager software. Ratios were calculated as the mean of $(1 + E)^{-\Delta\Delta C_t}$ values, where E is the efficiency, and $\Delta\Delta C_t$ is the target gene expression of treated cells compared to untreated cells, corrected to the expression of 2 reference genes *gapdh* and *β -actin*. Results were expressed as the fold-factor induction: ratio of $(1 + E)^{-\Delta\Delta C_t}$ of treated cells/ $(1 + E)^{-\Delta\Delta C_t}$ of untreated cells.

CD4+ T-cell proliferation and polarization experiments

MoDC were stimulated with NiSO_4 (500 μM) for 4 hrs, washed to eliminate any residual nickel in the media and kept for 20 hrs for optimal MoDC maturation, in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (Biowest, France), 1% sodium pyruvate and 1% penicillin/streptomycin (Gibco Invitrogen). Purified allogeneic total CD4+ T-cells (10^6 cells/ml, Miltenyi Biotec, Germany) were co-cultured with nickel-activated MoDC (10^5 cells/ml), in round-bottom 96-well plates. The purity (>95%) of CD4+ T-cells was confirmed by flow cytometry. In some wells, human anti-IL-27 neutralizing Ab or control goat IgG (10 $\mu\text{g/ml}$; R&D Systems, Minneapolis) were added to NiSO_4 -activated MoDC 1 h before the co-culture. At day 5, cytokine production was analyzed as described below. In some experiments, allogeneic CD4+ T-cells were labelled with 0.5 μM CFSE (Invitrogen) and then co-cultured with MoDC (10^5 cells/ml). Five days later, CD4+ T-cell proliferation was analyzed by flow cytometry and expressed as the percentage of CFSE low cells.

Quantification of cytokine production

Human IL-27 ELISA assays were performed on culture supernatants of MoDC treated or not with nickel, and measured in duplicate, according to the manufacturer's instructions (R&D Systems, Minneapolis). To assess T-cell polarization, MoDC/CD4+ T-cell coculture supernatants, obtained after 5 days of coculture, were measured in duplicate for cytokine

contents. IL-22, IL-17A, IFN- γ , IL-5, IL-13, IL-9 and IL-10 were quantified using MSD multiplex assay, following manufacturer's instructions (Meso Scale Discovery, Gaithersburg, MD, USA).

Statistical analysis

Data are expressed as mean $\pm$ SD. Differences between groups were evaluated with the Wilcoxon signed rank test or the Mann–Whitney U test (GraphPad, La Jolla, CA). The p values <0.05 were considered to denote statistical significance.

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109 RESULTS

110 Nickel induces the production of IL-27 by human MoDC.

111 We first measured the production of IL-27 by human MoDC after nickel stimulation. iDC were
112 treated or not (control) with NiSO₄ or NiCl₂, at 500 µM, for 24 hrs. Both metals induced the
113 production of IL-27 in the same manner with an average of 750 pg/ml (**Fig. 1a**). In the same
114 conditions, an average of 6600 pg/ml was measured after LPS stimulation (25 ng/ml) (**Fig. 1a**).
115 NiSO₄ induced the production of IL-27 in a concentration-dependent manner with a significant
116 production from 300 µM (**Fig. 1b**). We then studied mRNA expression of IL-27 subunits in
117 nickel-activated MoDC. Indeed, nickel upregulated the expression of the two subunits of IL-27:
118 IL-27p28 (**Fig. 1c**) and *ebi3* (**Fig. 1d**) at 4 hrs post-stimulation. In contrast to the early induction
119 and rapid decay of *il-23p19* mRNA, the specific subunit for IL-23, *il-27p28* mRNA expression
120 was extended and followed the same kinetic as *il-12p40* mRNA expression, the common subunit
121 for IL-12p70 and IL-23 (**Fig. 1e**). As for *ebi3* mRNA, its expression was more stable during
122 time. Low levels of *il-12p35* mRNA were detected after nickel stimulation as previously
123 described¹⁴ and shown in **Fig. 1e**. IL-27 produced by nickel-activated MoDC followed a delayed
124 kinetic in comparison to nickel-induced IL-23 production (**Fig. 1f**).

125 Neutralization of IL-27 produced by NiSO₄-treated MoDC increases the production of IL-22 by human CD4⁺ T-cells

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128 We next examined the functionality of the IL-27 produced by NiSO₄-activated MoDC and we
129 addressed its role on human CD4⁺ T-cell proliferation and polarization using allogeneic co-
130 cultures between MoDC and total CD4⁺ T-cells. MoDC were treated, with or without NiSO₄, in

the presence or absence of an anti-IL-27 antibody or its isotype control, and then co-cultured with allogeneic CD4⁺ T-cells stained with CFSE. Cytokine production of CD4⁺ T-cells was also evaluated 5 days later by quantifying cytokines in cell culture supernatants using an MSD multiplex assay. NiSO₄-activated MoDC augmented the proliferation of CD4⁺ T-cells (**Fig. 2**) and the secretion of IL-22, IL-17, IFN- γ , IL-5, IL-13 and IL-9 illustrating a mix T-cell response (**Fig. 3**). The addition of IL-27 neutralizing Ab did not modify CD4⁺ T-cell proliferation (**Fig. 2**) but significantly augmented the secretion of IL-22 by CD4⁺ T-cells (**Fig. 3a**). An increase in IL-17 production was also observed but did not reach statistical significance (**Fig. 3b**). These results suggested that IL-27 produced by NiSO₄-activated MoDC modulated Th22/Th17 polarization essentially by controlling IL-22 production by CD4⁺ T-cells.

The TLR4, p38 MAPK and NF- κ B pathways contribute to NiSO₄-induced production of IL-27.

To investigate if the effect of nickel on the IL-27 secretion was mediated by the TLR4 signaling pathway, we performed TLR4 blocking experiments. Blocking TLR4 induced a concentration-dependent decrease in IL-27 production at 24 hrs by nickel-treated MoDC (**Fig. 4a**). Moreover, inhibition of p38 MAPK (SB203580) or NF- κ B (BAY 11-7085) pathways induced a significant decrease in the production of IL-27 at 24 hrs (**Fig. 4b**). Likewise, these pathways mediated the expression of il-27p28 mRNA subunits (**Fig. 4c and e**). The expression of ebi3 mRNA was decreased with TLR4 blocking antibody and the inhibition of the NF- κ B pathway (**Fig. 4d and f**) but was not modified by the p38 MAPK inhibitor (**Fig. 4f**).

The Jak-STAT pathway is implicated in IL-27 production.

We previously showed that the Jak-STAT pathway differentially regulates the IL-23/IL-12p70 balance in human MoDC treated with nickel. Here, we asked whether Jak-STAT activation was

155 also required for IL-27 production. The inhibition of the Jak-STAT pathway induced a decrease
156 in the production of IL-27 at 24 hrs (**Fig. 5a**). We next evaluated the effect of Jak-STAT
157 inhibition on IL-27 subunits regulation. The inhibition of Jak-STAT pathway in nickel-activated
158 MoDC induced a decrease in the expression of *il-27p28* mRNA (**Fig. 5b**) but did not modified
159 the expression of *ebi3* mRNA (**Fig. 5c**). Simultaneously, the concomitant presence of NiSO₄ and
160 IFN- γ , a potent activator of the Jak-STAT pathway, induced an increase in the production of the
161 IL-27 (**Fig. 5d**) due to a significant increase in the expression of *il-27p28* mRNA in NiSO₄-
162 treated MoDC (**Fig. 5e**) while not modifying the expression of *ebi3* mRNA (**Fig. 5f**). Taken
163 together, these data suggested that the Jak-STAT pathway differentially regulated the expression
164 of IL-27 subunits in NiSO₄-activated MoDC.

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176 DISCUSSION

177 IL-27 was first described by Pflanz *et al*¹⁵ as a heterodimeric cytokine consisting of two protein
178 subunits: EBV-induced gene 3 (ebi3) and p28 or il-27p28. Both subunits of IL-27 were found to
179 be expressed in chronic lesional allergic eczematous skin¹⁶ and administration of IL-27
180 contributed to the amelioration of delayed-type hypersensitivity model in mice¹⁷. In order to
181 further understand the implication of IL-27 in ACD and since DC are believed to play a major
182 role in immune regulation, the objective of this study was to understand if nickel, the most
183 common metallic sensitizer, can induce the production of IL-27 by human MoDC and to
184 evaluate its role and its regulation.

185 To our knowledge, this is one of the first studies to report the ability of nickel to induce the
186 production of IL-27 in human MoDC. Interestingly, Tomić *et al*¹⁸ showed upregulation of IL-
187 27 in human MoDC treated with rapidly solidified nickel-titanium ribbons. Nevertheless, a direct
188 link between nickel and IL-27 production could not be concluded from their study since authors
189 showed insignificant nickel release in culture medium. The production of IL-27 by LPS, used as
190 a positive control, is in accordance with a previous report¹⁹ and different TLR agonists were also
191 able to induce IL-27 by human DC or macrophages^{20–22}. Simultaneously, nickel induced the
192 expression of both IL-27 subunits: il-27p28 and ebi3 with different kinetics suggesting the
193 implication of different signaling pathways in IL-27 subunits expression. Moreover, in
194 comparison to the early production of IL-23¹⁴ and the early expression of its specific subunit, the
195 expression of *il-27p28* mRNA was more delayed and increased with time. However, the

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3 196 expression of *ebi3* mRNA seemed to be more stable suggesting its role as a limiting factor in IL-
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5 197 27 production. Despite similar clinical reactions in the majority of patients with ACD, distinct
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7 198 immune polarizations characterized different contact sensitizers. For instance, nickel
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9 199 demonstrated a strong Th1/Th17 and Th22 polarization^{23,24}. While IL-12p70 promotes Th1 cells
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11 200 polarization, IL-23 promotes Th17 cells development. However, IL-27 is a cytokine with
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13 201 pleiotropic properties with both pro-inflammatory and anti-inflammatory proprieties¹³. IL-27
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15 202 was initially described as a pro-inflammatory cytokine that promotes Th1 responses¹⁵. However,
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17 203 in our experiment, the inhibition of IL-27 produced by nickel-activated MoDC did not modify
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19 204 the Th1 response. This discrepancy could be due to the presence of an optimal concentration of
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21 205 IL-12p70 that compensates the inhibition of IL-27. Of all the major cytokines produced by
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23 206 activated CD4+ T cells, IL-27 was able to regulate specifically the production of IL-22 since IL-
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25 207 27 neutralization induced an increase in IL-22 production by allogeneic CD4+ T-cells without
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27 208 modifying T-cell proliferation. An increase of IL-17 production was also observed in our study
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29 209 but did not reach statistical significance. Our results are in accordance with previous reports
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31 210 demonstrating the inhibitory effect of IL-27 on IL-22 production by human CD4+ T cells^{25,26} and
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33 211 on Th17 cells in general by downregulating RORC expression in an IL-10-independent
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35 212 manner^{27,28}. Since IL-22 is implicated in dermal inflammation^{29,30} and IL-22 serum levels are
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37 213 significantly higher in patients affected by nickel-induced ACD compared with controls²⁹, our
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39 214 results support a regulatory role of IL-27 in ACD. We therefore propose a model, in which the
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41 215 balance of IL-27 vs IL-23 signaling in CD4+ T cells is critical for the development of pathogenic
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43 216 Th17/Th22 cells. Taking into consideration that IL-27 production by nickel-treated MoDC is
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45 217 more delayed compared to IL-23, we suggest that dendritic cells activated by nickel can promote,
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47 218 in an early manner, the development of IL-17 producing CD4+ T-cells that contribute to skin
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pathology¹⁴. The delayed production of IL-27 by nickel-activated dendritic cells may play a role in a delayed regulation of this inflammation by targeting IL-22 production.

In the last part of our study, we were interested in the signaling pathways leading to nickel-induced IL-27 by MoDC. In this context, it is important to decipher the mechanisms that regulate the balance between the different members of the IL-12 family. Since nickel is known to be a TLR4 agonist⁵, we investigated the implication of the TLR4 pathway in nickel-induced IL-27 production. As evidenced before for IL-23, IL-12p40 and IL-12p70¹⁴, our result clearly showed the implication of TLR4 in IL-27 production. Moreover, we have previously shown that NiSO₄ can activate MAPK and NF-κB pathways in human DC^{10–12,31}. Here we showed that IL-27 production in NiSO₄-activated MoDC was also dependent on p38 MAPK and NF-κB pathways as previously found with IL-23, IL-12p40 and IL-12p70¹⁴. However, only the NF-κB pathway was involved in *ebi3* mRNA expression as previously shown with studies performed on LPS-activated DC or macrophages³². Thus, the synthesis of IL-12 family members in nickel-activated MoDC can be differentially regulated downstream of the TLR4 pathway (**Figure 6**).

On the other hand, we found that the production of IL-27 was partially mediated by the Jak-STAT pathway. Although the regulation of the *ebi3* subunit seemed to be independent of this pathway, the expression of *il-27p28* mRNA was decreased following Jak-STAT inhibition. Simultaneously and as expected, the activation of the Jak-STAT pathway when adding IFN-γ to NiSO₄ stimulation induced the expression of *il-27p28* mRNA without modifying *ebi3* mRNA expression. Thus, *ebi3* and *il-27p28* were differentially regulated by the Jak-STAT pathway. Among the other members of IL-12 cytokine family, *il-12p35* was highly dependent on the Jak-STAT pathway. However, *il-23p19* and *il-12p40* were both inhibited by this pathway¹⁴. Taken together, these results showed important differences in the transcriptional regulation of the

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3 242 different polypeptide chains of the IL-12 family members by the Jak-STAT pathway in human
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5 243 MoDC (**Figure 6**). Therefore we hypothesized that in nickel-allergic patients, the inhibition of
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7 244 the Jak-STAT pathway in the skin dendritic cells by some natural co-exposed products (ie
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10 245 terpens³³) for example, may prevent Th1 cells development in favor of Th17/Th22 polarization.
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12 246 In summary, we showed that nickel can induce IL-27 production in human MoDC regulating IL-
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14 247 22 production by human CD4+ T cells. Simultaneously, nickel induced the expression of *il*-
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16 248 *27p28* and *ebi3* mRNA. However, in contrast to the IL-23 subunits, *il*-27p28 and *ebi3* were
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18 249 differentially regulated by the TLR4 and Jak-STAT downstream pathway. Although both
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20 250 subunits were positively regulated by the TLR4 and NF-κB pathways, only *il*-27p28 was also
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22 251 dependent on p38 MAPK and Jak-STAT pathway. Therefore, elucidating the mechanism of
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24 252 nickel-induced regulation of the IL-12 cytokine family in DC will further expand our
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26 253 understanding of ACD provoked by nickel exposition.
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Conflicts of interest: The authors state no conflict of interest.

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Abbreviations: ACD: Allergic contact dermatitis; DC: Dendritic cells; Ebi3: Epstein-barr virus induced 3; Jak: Janus Kinase; MAPK: Mitogen-activated protein kinase; MoDC: Monocyte-derived dendritic cells; NiSO₄: Nickel sulfate; STAT: Signal transducers and activators of transcription; Th: T-helper.

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FIGURE 1. Effect of nickel on the production of IL-27 and the expression of its subunits in human MoDC. **(a)** Immature DC were treated or not with 500 μ M of NiSO₄, NiCl₂ or 25 ng/ml of LPS for 24 hrs. **(b)** Immature DC were treated with different concentrations of NiSO₄ for 24 hrs. **(a and b)** ELISA was performed to measure IL-27 production. Values are presented as mean $\pm$ SD of 4 independent experiments. **(c and d)** Immature DC were treated or not with 500 μ M of NiSO₄, NiCl₂ or 25 ng/ml of LPS for 4 hrs. *il-27p28* **(c)** and *ebi3* **(d)** mRNA levels were quantified by qRT-PCR. Results are expressed as fold factor compared with untreated samples and corrected by the expression of *β -actin* and *gapdh*. Values are presented as mean $\pm$ SD of 4 independent experiments. **(e)** Kinetics of *il-23p19*, *il-12p40*, *il-27p28*, *ebi3* and *il-12p35* mRNA expression following DC treatment with 500 μ M of NiSO₄. Results are expressed as fold factor compared with untreated samples and corrected by the expression of *β -actin* and *gapdh*. Values are presented as mean $\pm$ SD of 3 independent experiments. **(f)** Kinetics of IL-27 and IL-23 production following DC treatment with 500 μ M of NiSO₄. Values are presented as mean $\pm$ SD of 3 independent experiments. *p $\leq$ 0.05, Mann-Whitney.

FIGURE 2. Anti-IL-27 Ab did not modify the ability of NiSO₄-activated MoDC to induce CD4⁺ T cell proliferation. CFSE-loaded CD4⁺ T cells were cultured with allogeneic untreated-MoDC or with different conditions at ratio 10/1. On day 5, the percentage of CFSE^{low} cells was determined by flow cytometry. A gating strategy was used to exclude dead cells and debris, based on the FSC/SSC parameters, and the fluorescence acquisition was performed counting 10

000 cells within the gate. CFSE fluorescence intensity is presented on the x-axis on a log scale and cell count is displayed on the y-axis. Gates for proliferating cells (CFSE low) were set at 99% of non-proliferating CD4⁺ T-cells in control sample (CD4⁺ T-cell alone). **(a)** Cumulative data from 4 experiments. Values are presented as mean $\pm$ SD. * $p \leq 0.05$, Mann-Whitney. **(b)** One representative of 4 experiments is shown

FIGURE 3. Inhibition of IL-27 produced by nickel-activated MoDC modulates CD4⁺ T-cell polarization. Total CD4⁺ T-cells were co-cultured with MoDC, treated or not with NiSO₄ (500 μ M), in the presence or absence of an anti-IL-27 antibody (10 μ g/ml) or its isotype control (10 μ g/ml). On day 5, cytokines were quantified in co-culture supernatants using a Multiplex assay. The results are presented in box and whisker diagrams. The solid line within each box represents the median value. The top and bottom of the whisker shows the maximum and the minimum values. ** $p \leq 0.01$, Wilcoxon signed rank test.

FIGURE 4. Production of IL-27 in NiSO₄-activated MoDC is TLR4, p38MAPK and NF- κ B-dependent. **(a)** At day 5, immature DC were pretreated or not (control) for 1 h with an anti-TLR4 or isotype control antibodies (1, 5 and 10 μ g/ml). Cells were then treated or not with NiSO₄ (500 μ M) for 24 hrs. **(b)** At day 5, immature DC were pretreated or not (control) for 30min with inhibitor of p38MAPK pathway (SB203580, 20 μ M) or for 1 h with either DMSO or with inhibitor of NF- κ B pathway (BAY 11-7085, 3 μ M). Cells were then treated or not with NiSO₄ (500 μ M) for 24 hrs. **(a and b)** ELISA was performed after 24 hrs of stimulation. Values are presented as mean $\pm$ SD of 4 independent experiments. * $p \leq 0.05$, Mann-Whitney. **(c and d)** At day 5, immature DC were pretreated or not (control) for 1 h with an anti-TLR4 or isotype control antibodies (10 μ g/ml). Cells were then treated or not with NiSO₄ (500 μ M) for 4 hrs. *il-27p28* **(c)**

and *ebi3* (**d**) mRNA levels were quantified by qRT-PCR. (**e and f**) At day 5, immature DC were pretreated or not (control) for 30 min with inhibitor of p38MAPK pathway (SB203580, 20 μ M) or for 1 h with either DMSO or with inhibitor of NF- κ B pathway (BAY 11-7085, 3 μ M). Cells were then treated or not with NiSO₄ (500 μ M) for 4 hrs. *il-27p28* (**e**) and *ebi3* (**f**) mRNA levels were quantified by qRT-PCR. (**c-f**) Results are expressed as fold factor compared with untreated samples and corrected by the expression of *β -actin* and *gapdh*. Values are presented as mean $\pm$ SD of 4 independent experiments. * $p \leq 0.05$, Mann-Whitney.

FIGURE 5. Role of the Jak-STAT pathway in NiSO₄-induced IL-27 production by MoDC. At day 5, immature DC were pretreated or not (control) with either DMSO or with the inhibitor of the Jak-STAT pathway (Jak Inhibitor I, 0.5 μ M). Cells were then treated or not with NiSO₄ (500 μ M) for 24 hrs (**a**). ELISA was performed after 24 hrs of stimulation. Values are presented as mean $\pm$ SD of 4 independent experiments. (**b and c**) At day 5, immature DC were pretreated or not with the inhibitor of the Jak-STAT pathway (Jak Inhibitor I, 0.5 μ M). Cells were then treated or not with NiSO₄ (500 μ M) for 4 hrs. *il-27p28* (**b**) and *ebi3* (**c**) mRNA levels were quantified by qRT-PCR. Results are expressed as fold factor compared with untreated samples and corrected by the expression of *β -actin* and *gapdh*. DC were treated or not with NiSO₄, in addition or not of IFN- γ (1,000 U/ml) for 24 hrs (**d**) or 4 hrs (**e and f**). (**d**) ELISA was performed to measure IL-27 production. *il-27p28* (**e**) and *ebi3* (**f**) mRNA levels were quantified by qRT-PCR. Results are expressed as fold factor compared with untreated samples and corrected by the expression of *β -actin* and *gapdh*. Values are presented as mean $\pm$ SD of 4 independent experiments. * $p \leq 0.05$, Mann-Whitney.

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FIGURE 6. Regulation of the IL-12 cytokine family by nickel in human dendritic cells. We previously showed that nickel induced the production of IL-12p40, IL-23 in MoDC but low levels of IL-12p70 were detected. Here we showed that nickel was also able to induce the production of IL-27. The effect of nickel on these cytokines correlated with the expression of their subunits. Whereas IL-12p70 drives Th1 type response and IL-23 drives Th17 type response, IL-27 seems to downregulate IL-22 production. All the subunits of IL-12p70, IL-23 and IL-27 are positively regulated by the TLR4, p38 MAPK and NF-κB pathways with the exception of ebi3 which was not regulated by p38 MAPK. Among the IL-12 cytokine family, IL-12p70 production was highly dependent on Jak-STAT signaling. However, IL-23 and IL-12p40 were inhibited by this signaling pathway. As for IL-27, its production was partially mediated by the Jak-STAT signaling.

Figure 1

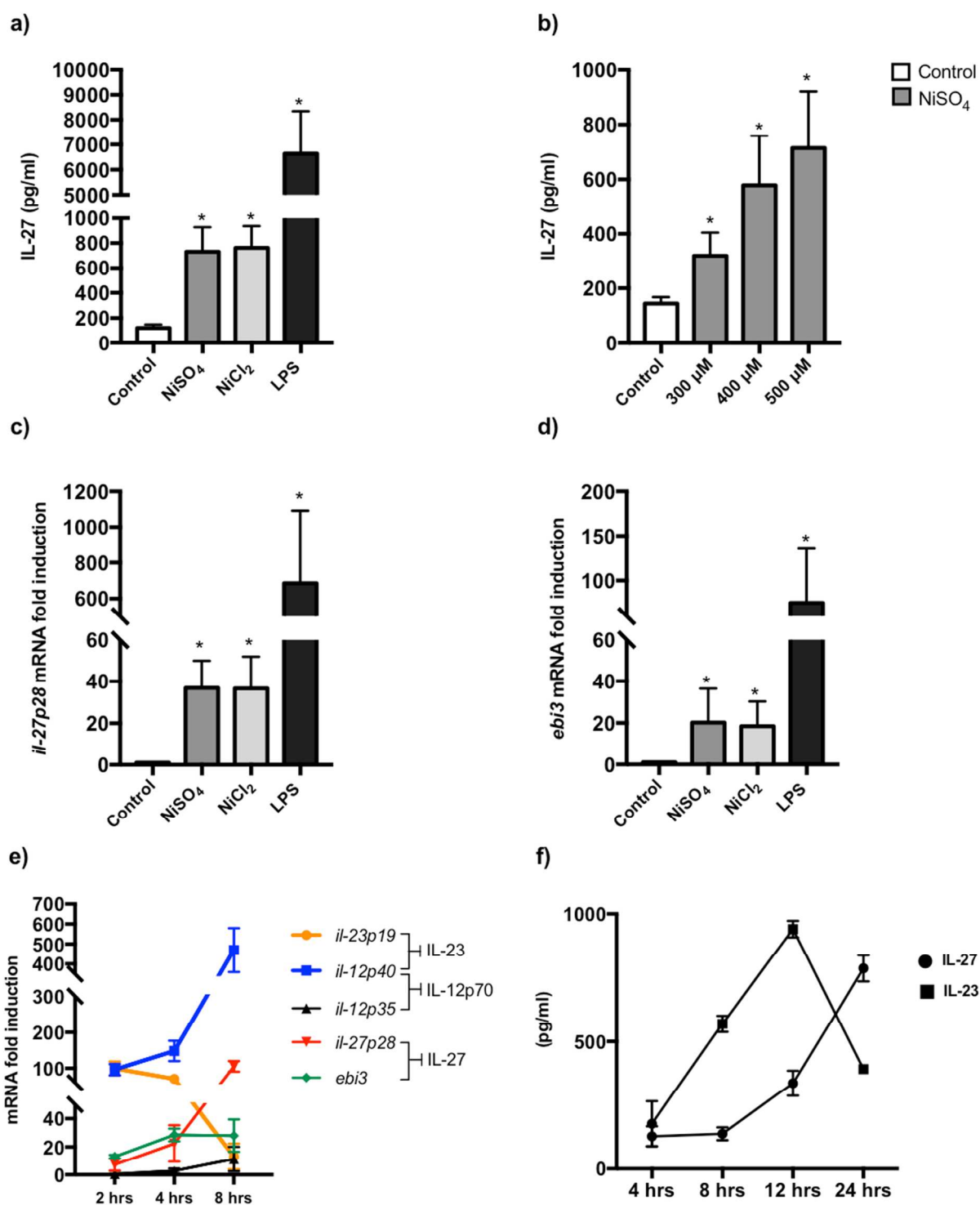


Figure 2

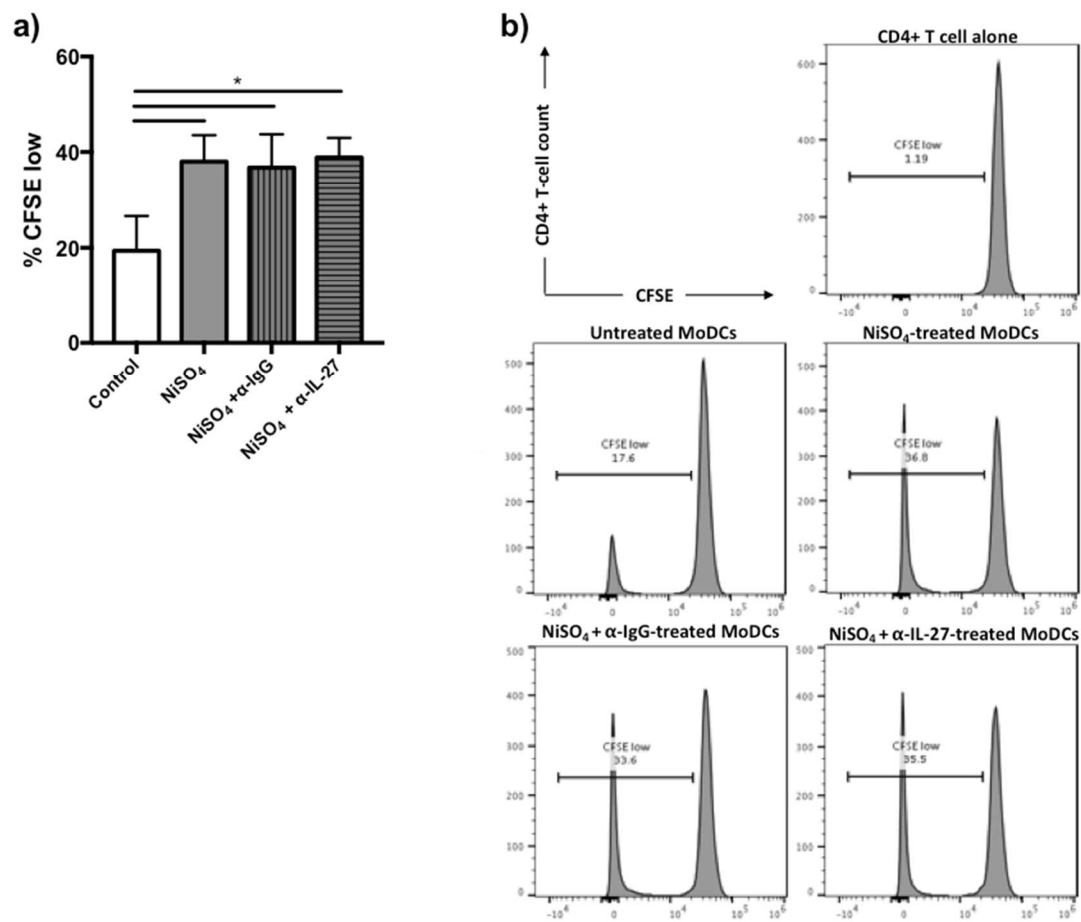


Figure 3

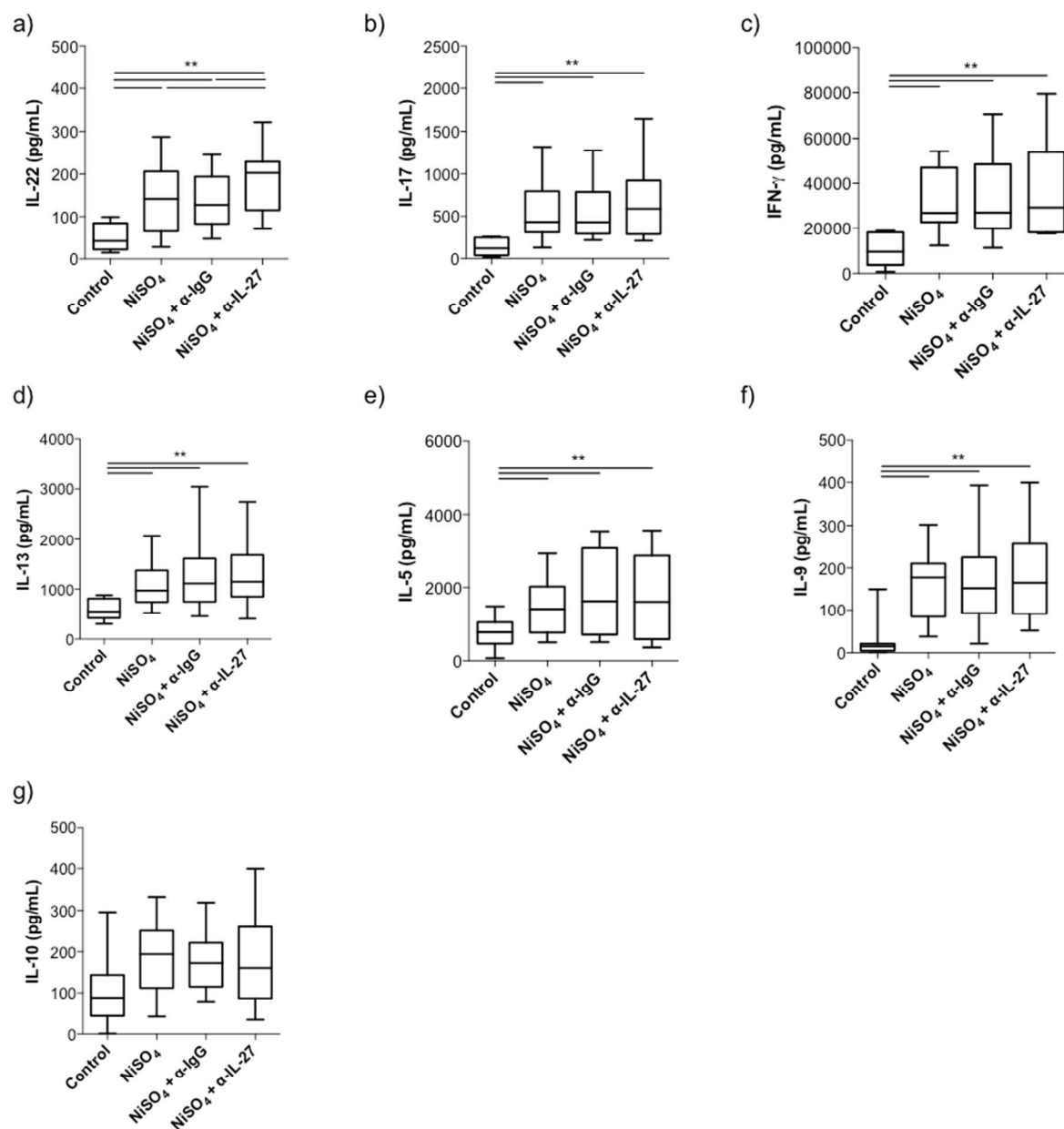


Figure 4

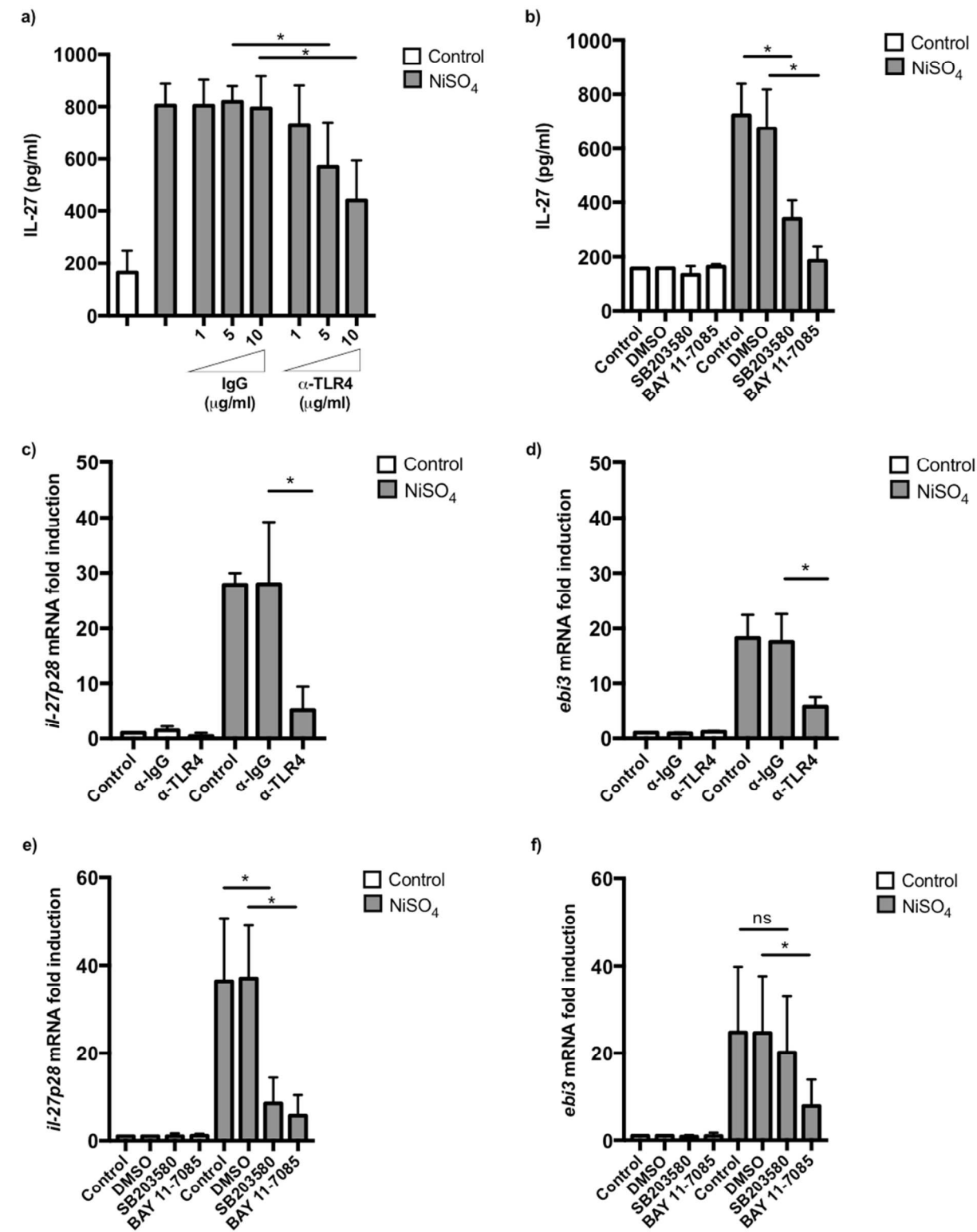


Figure 5

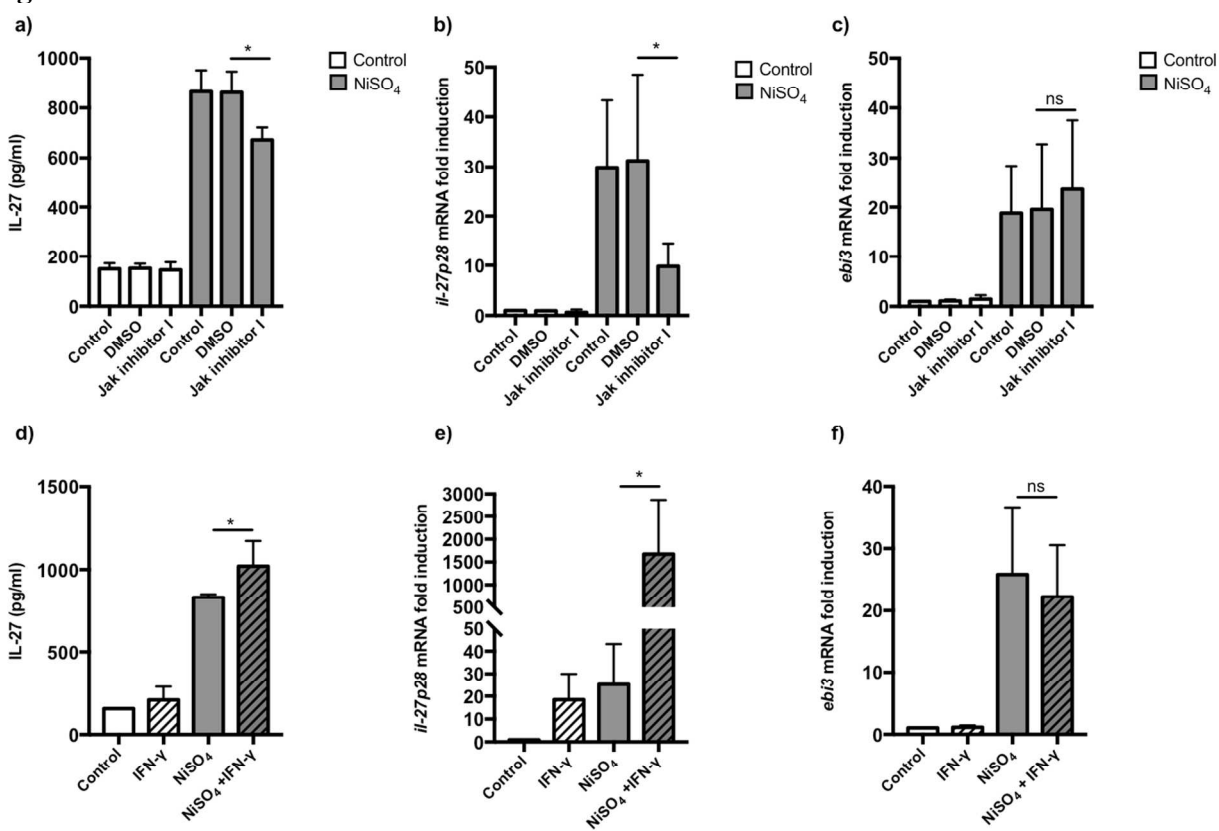


Figure 6

