



# Phenotypic alterations and cytokine production in THP-1 cells in response to allergens

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## Abstract

In the induction phase of allergic contact hypersensitivity, dendritic cells (DCs), including Langerhans cells (LCs) present in epidermis, can trigger an efficient T cell response once they have matured in response to an allergen. Upon maturation, DCs have been shown to induce expression of several surface molecules and the up-regulation of cytokine production. We have previously shown that THP-1 cells, human acute monocytic leukemia cell line, can discriminate between allergens and irritants by measuring expression of surface markers, CD86 and CD54, following chemical exposure. At the same time, we have also reported that augmented expression of HLA and CD80, and production of IL-1 $\beta$  were up-regulated in THP-1 cells when treated with an allergen, 2,4-dinitrochlorobenzene (DNCB). In the present study, we first evaluated whether THP-1 cells induced the phenotypic changes and the production of cytokines, which are observed in the process of DC maturation, when treated with two known allergens, DNCB and nickel sulfate (NiSO<sub>4</sub>), and one irritant (sodium lauryl sulfate (SLS)). Exposure to DNCB and NiSO<sub>4</sub> induced significant augmentation of CD40 and CD83 expression as well as CD86 and CD54. Also, TNF- $\alpha$  and IL-8 secretion were markedly induced by DNCB and NiSO<sub>4</sub> in a dose-dependent manner. In addition, DNCB and NiSO<sub>4</sub> augmented CD1a expression and production of IL-6, respectively. On the contrary, SLS did not change any of these markers. We then evaluated a series of chemicals, including six known allergens (e.g., hydroquinone (HQ)) and two non-allergens (e.g., methyl paraben (MP)), in order to investigate the potential increase of CD86, CD54, CD40, and CD83 expression on THP-1 cells, and production of TNF- $\alpha$  and IL-8. Indeed, all tested allergens, except eugenol (EU), caused significant increased changes in at least four of the analyzed six markers, while non-allergens did not induce any changes. EU significantly augmented CD86, CD54 and CD40 expression. These results revealed that the wide variety of responses to allergens in THP-1 cells may emulate allergen-induced maturation processes of DCs. It is suggested that THP-1 cells, which could develop several DC-like properties, are suitable for identifying sensitizing potential of chemicals.

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**Keywords:** Contact hypersensitivity; THP-1 cell; Surface marker expression; Cytokine production; Langerhans cell

## 1. Introduction

Many low molecular weight compounds can give rise to the phenomenon of allergic contact dermatitis. The guinea pig maximization test (Magnusson and Kligman, 1969) and the local lymph node assay (LLNA) (Kimber et al., 1986) have been commonly used to assess the sensitizing potential of chemicals. Meanwhile, there has been considerable interest to develop an in vitro predictive method for identification of chemical allergens from the point of view of animal welfare, cost reduction and time involvement. Many researchers have tried several approaches in developing an

**Abbreviations:** ATCC, American Type Culture Collection; DCs, dendritic cells; DMSO, dimethyl sulfoxide; DNCB, 2,4-dinitrochlorobenzene; EU, eugenol; FA, formaldehyde; HQ, hydroquinone; h-CLAT, human Cell Line Activation Test; HC, hydroxycitronellal; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-6, interleukin-6; IL-8, interleukin-8; LA, lactic acid; LC, Langerhans cell; LPS, lipopolysaccharide; mAb, monoclonal antibody; MP, methyl paraben; MFI, mean fluorescence intensity; MHC class II, class II major histocompatibility complex; NiSO<sub>4</sub>, nickel sulfate; PAA, phenylacetaldehyde; PG, propyl gallate; PI, propidium iodide; SLS, sodium lauryl sulfate; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

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in vitro skin sensitization test. One approach is to develop cellular-based methods (Ryan et al., 2001).

Langerhans cells (LCs) play a critical role in the induction phase of allergic contact hypersensitivity. Following exposure to chemical allergens, LCs are capable of internalizing and processing them. Upon antigen capture, LCs differentiate, mature, and migrate to the draining lymph nodes where they present the allergens to naïve T cells and trigger their proliferation (Hart, 1997; Banchereau and Steinman, 1998). During the course, LC maturation is characterized by the up-regulation of major histocompatibility complex (MHC) class II and expression of co-stimulatory molecules, such as CD86, CD54, CD40, and CD83 (Ozawa et al., 1996; Verrier et al., 1999) as well as cytokine production like interleukin (IL)-1 $\beta$  and IL-6 (Enk and Katz, 1992; Cumberbatch et al., 1996).

To date, the use of LCs has been restricted in the development of an in vitro skin sensitization test due to the difficulty of obtaining sufficient number of LCs and to the spontaneous maturation of LC once cultured. Therefore, LC-like DCs have recently been derived from monocytes (Mo-DC) (Sallusto and Lanzavecchio, 1994) and CD34+ progenitor cells (CD34-DC) (Caux et al., 1992) with some cytokines. Many attempts have demonstrated that chemical allergens have directly induced in vitro phenotypic alterations (e.g. HLA-DR, CD86, CD54) and cytokine production (e.g. tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )), IL-1 $\beta$ , IL-8 of Mo-DC or CD34-DCs (Aiba et al., 1997, 2003; Coutant et al., 1999; De Smedt et al., 2001, 2002; Staquet et al., 2004). These studies suggested a possibility of being able to reproduce in vitro the key steps involved in DC maturation. However, in these cell models, donor-to-donor variations in response to allergens have been reported for cell surface molecule expression (Coutant et al., 1999; Tuschl et al., 2000). Beside individual variability, the low abundance of DCs in the source represents a major technical hurdle for routinely employing these cells in skin sensitization tests. In order to eliminate these problems, we focused on the use of THP-1 cells, human acute monocytic leukemia cell line, which are more homogeneous and commercially available. In addition, the up-regulation of surface molecules, CD86 and CD54, as well as increased internalization of MHC class II molecules were observed following exposure to chemical allergens in THP-1 cells (Ashikaga et al., 2002; Yoshida et al., 2003). Furthermore, we have recently developed an in vitro predictive method for assessing sensitizing potential by measuring changes in CD86 and CD54 expression on THP-1 cells following exposure to chemicals (human Cell Line Activation Test; h-CLAT) (Ashikaga et al., 2006; Sakaguchi et al., 2006). To confirm the potential of THP-1 cells as LC surrogates, we evaluated whether THP-1 cells can display allergen-induced changes in a similar fashion as those shown to provoke DCs including LCs. The response was assessed by measuring changes in markers reflecting DC maturation (CD86, CD54, CD40, CD83, CD58, CD1a, TNF- $\alpha$ , IL-6 and IL-8).

## 2. Materials and methods

### 2.1. Cell line and medium

THP-1 cells from American Type Culture Collection (ATCC, Manassas, VA, USA) were cultured in RPMI1640 containing 25 mM HEPES buffer and L-glutamine (Invitrogen Corp., Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA), 0.05 mM 2-mercaptoethanol and 1% penicillin–streptomycin (Invitrogen Corp., Carlsbad, CA, USA).

### 2.2. Chemical treatment of THP-1 cells

Cells ( $1 \times 10^6$ ) were cultured for 24 h in 24-well plates in 1 mL of culture medium with or without the chemical. Cells were treated at various concentrations that exhibited 70–95% cell viability. Test allergens were: 2,4-dinitrochlorobenzene (DNCB), nickel sulfate hexahydrate (NiSO<sub>4</sub>), hydroquinone (HQ), propyl gallate (PG), formaldehyde (FA), eugenol (EU), phenylacetaldehyde (PAA) and hydroxycitronellal (HC). Test non-allergens were sodium lauryl sulfate (SLS), methyl paraben (MP) and lactic acid (LA). All test chemicals and lipopolysaccharide (LPS) from *Salmonella abortus equi* were purchased from Sigma–Aldrich (St. Louis, MO, USA). NiSO<sub>4</sub>, HQ, FA, SLS, LA and LPS were initially prepared in physiological saline, while DNCB, PG, PAA, EG, HC and MP were dissolved in dimethylsulfoxide (DMSO; Sigma–Aldrich) and added to the medium. The final concentration of DMSO in culture media was less than 0.2%. At least three independent experiments were done for each chemical tested.

### 2.3. Phenotypic analysis by flow cytometry

After 24 h treatment with chemicals, cells were recovered and expression of cell surface markers was analyzed by flow cytometry. Cell staining was performed using the following FITC-conjugated monoclonal antibodies (mAbs): anti-human CD54 (clone; 6.5B5) from DAKO (Glostrup, Denmark) and anti-human CD86 (clone; Fun-1), anti-human CD40 (clone; 5C3), anti-human CD83 (clone; HB15e), anti-human CD1a (clone; HI149) and anti-human CD58 (clone; 1C3) from BD PharMingen (San Diego, CA, USA). Isotypic controls were mouse IgG1-FITC from DAKO and IgG1,  $\kappa$ -FITC, IgG2a,  $\kappa$ -FITC from BD PharMingen.

Cells were incubated with mAbs and appropriated isotypic controls for 30 min at 4 °C after blocking Fc receptor with Globlins Cohn fraction II, III (Sigma–Aldrich) for 10 min at 4 °C. After washing and resuspending with phosphate buffered saline supplemented containing 0.1% bovine serum albumin, stained cells were analyzed by FACSCalibur and CellQuest software (Beckton Dickinson, San Jose, CA, USA). Dead cells were gated out after staining by 0.625  $\mu$ g/mL propidium iodide (PI) solution and cell viability was determined. A total of 10,000 living cells were analyzed. Mean  $\pm$  S.D. was obtained for at least three independent experiments giving the Mean Fluorescence Intensity

(MFI). MFI was used as an indicator of surface marker expression.

#### 2.4. Analysis of cytokine production

Culture supernatants were recovered after 24 h exposure to various chemicals. The production of cytokines (TNF- $\alpha$ , IL-6 and IL-8) was measured by ELISA kit (R&D Systems, Minneapolis, MN, USA) using 96-well plates according to the manufacturer's instructions.

#### 2.5. Statistics

Student's *t*-test was used to evaluate a statistical significance between the vehicle (DMSO)-treated cells and the chemical-treated cell using MFI values in order to assess increased changes in analyzed surfaced markers. Increases in fluorescent intensity corresponded to an up-regulation of analyzed markers. *P*-value less than or equal to 0.05 were considered statistically significant.

### 3. Results

#### 3.1. Phenotypic alteration induced by DNCB, NiSO<sub>4</sub> and SLS

We examined various surface phenotypic changes on THP-1 cells following a 24 h exposure to DNCB, NiSO<sub>4</sub>

and SLS at three concentrations that exhibited 70–95% cell viability. Fig. 1a and b shows representative FSC/SSC data and fluorescence histograms of THP-1 cells that were untreated, or treated with vehicle (DMSO), DNCB (2.5  $\mu$ g/mL), NiSO<sub>4</sub> (85  $\mu$ g/mL), and SLS (45  $\mu$ g/mL), respectively. According to FSC/SSC data, the population of living cells exhibited a widespread distribution in both non-treated and chemical-treated cells. The flow cytometric patterns of DMSO-treated cells were quite comparable to those of non-treated cells. Therefore, DMSO had no effect on the induction of surface marker expression in our experiment. These histograms demonstrate that THP-1 cells expressed high levels of CD58 and moderate levels of CD86 and CD40. In contrast, CD54, CD83 and CD1a were expressed at low levels since the expression levels of these surface markers were almost equivalent to those of isotype control. DNCB (2.5  $\mu$ g/mL) up-regulated the expression of all surface markers except CD58. NiSO<sub>4</sub> (85  $\mu$ g/mL) up-regulated the expression of CD40 and CD83 as well as those of CD86 and CD54. SLS (45  $\mu$ g/mL) and DMSO only (vehicle) did not cause a change in expression of any surface marker. The extent of peak shifts in histograms did not relate to changes in the cell size because FSC MFI did not correlate with fluorescent intensity (e.g., FSC MFI values between non-treated and DNCB are equivalent).

Fig. 2 shows calculated MFI values for CD86, CD54, CD40, CD83, CD1a and CD58 expression after a 24 h exposure to DNCB, NiSO<sub>4</sub> and SLS. Table 1 summarizes

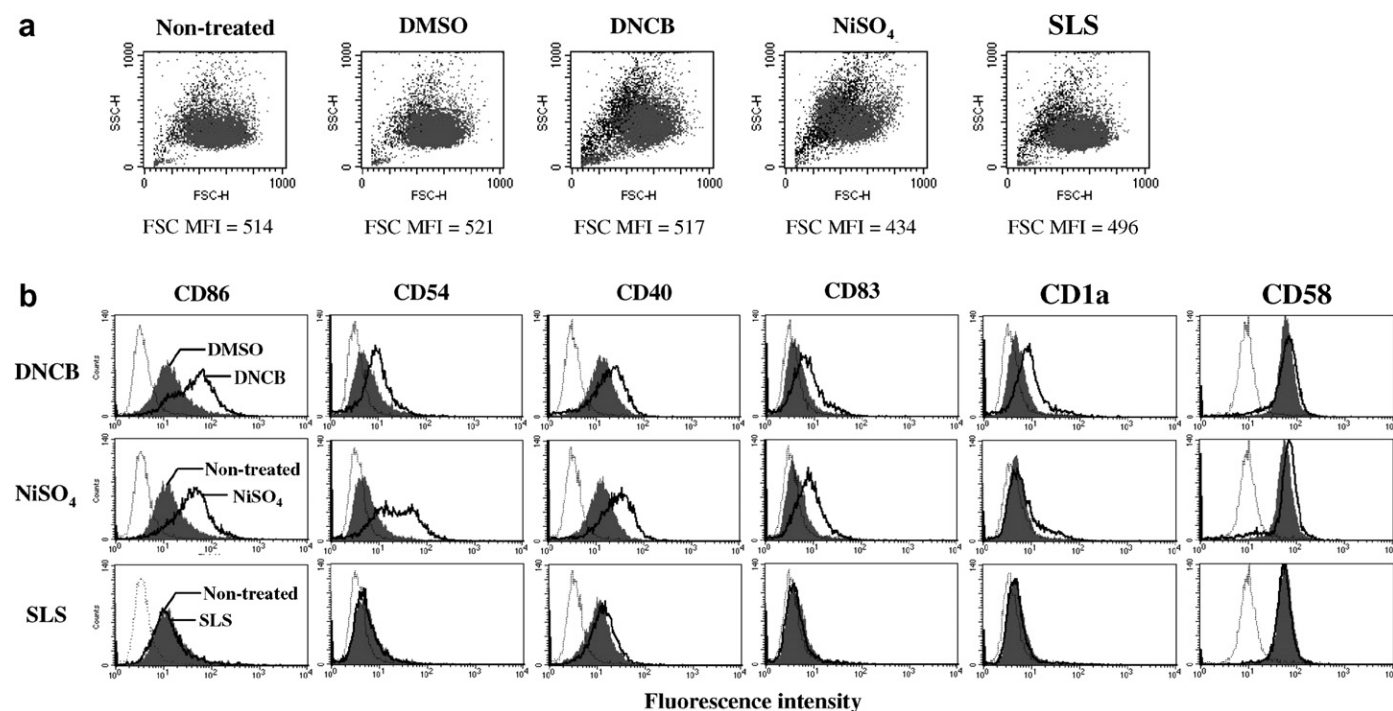


Fig. 1. Phenotypic alterations induced by DMSO (vehicle), DNCB (2.5  $\mu$ g/mL), NiSO<sub>4</sub> (85  $\mu$ g/mL), and SLS (45  $\mu$ g/mL). The data shown represent middle concentrations used in the study. These concentrations were selected as representative data that provide the maximum effect. (a) FSC/SSC data: living cells (gray dots) and dead cells (black dots), (b) Histograms for each surface marker evaluated in THP-1 cells: Isotype control (dotted line), vehicle-control (either DMSO or untreated; shaded peak), and chemical-treated (dark solid line). Since untreated control and DMSO-treated control gave the same results, untreated and DMSO-treated results were interchanged. These are representative data from three independent experiments. MFI = mean fluorescence intensity.

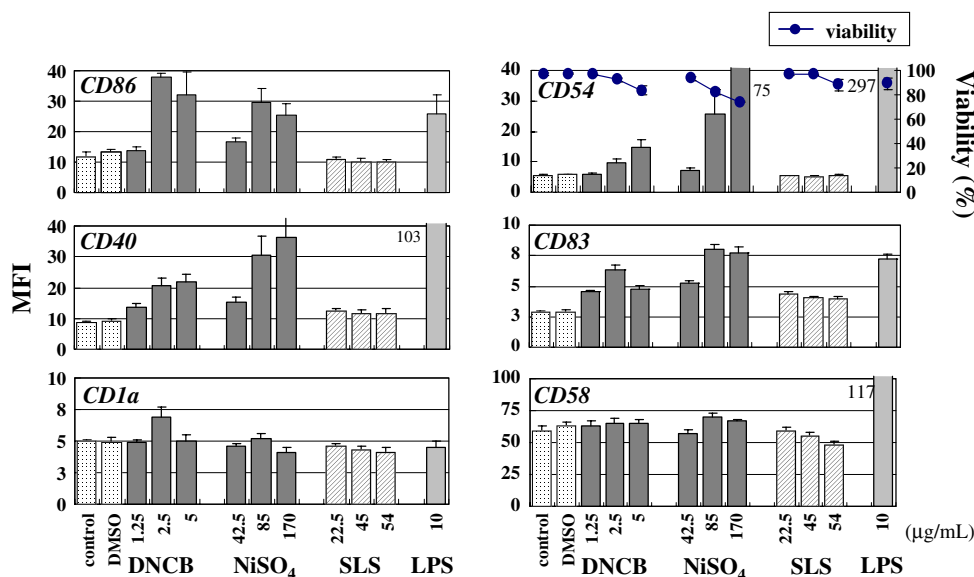


Fig. 2. Alterations of surface phenotypes by DNCB, NiSO<sub>4</sub>, SLS and LPS at the indicated concentrations. The results were expressed as MFI. Mean ± S.D. was given for at least three independent experiments. Statistical significance is reported in Table 1.

Table 1

Student's *t*-test was used to establish a statistical significance between MFI values for vehicle control cells and MFI values for chemical treated cells

|       | DNCB | NiSO <sub>4</sub> | HQ | PG | FA | PAA | EU   | HC | SLS  | MP   | LA   |
|-------|------|-------------------|----|----|----|-----|------|----|------|------|------|
| CD86  | **   | **                | *  | †  | *  | **  | *    | ** | n.s. | n.s. | n.s. |
| CD54  | **   | *                 | †  | *  | †  | **  | **   | *  | n.s. | n.s. | n.s. |
| CD40  | **   | **                | *  | ** | *  | **  | *    | ** | n.s. | †    | n.s. |
| CD83  | **   | **                | ** | ** | *  | *   | n.s. | ** | n.s. | n.s. | n.s. |
| TNF-α | **   | **                | *  | †  | *  | **  | n.s. | ** | n.s. | n.s. | n.s. |
| IL-8  | **   | **                | *  | *  | †  | *   | n.s. | ** | n.s. | n.s. | n.s. |

(\*\* *p* < 0.01; \* *p* < 0.05; † *p* < 0.1 compared with control; n.s.—not significant). Results were obtained from representative concentrations that induced a maximum effect.

the results of the statistical analysis between MFI values for vehicle control cells and MFI values for chemical-treated cells. These data demonstrated that when compared with control cells, DNCB and NiSO<sub>4</sub> statistically significantly augmented the expression of CD86, CD54, CD40 and CD83. The expression of CD1a was statistically significantly increased only by the treatment with DNCB (2.5 μg/mL). CD58 expression was not inducible by both DNCB and NiSO<sub>4</sub>. On the contrary, SLS did not change or slightly decreased expression of these markers. LPS (10 μg/mL) was used as a positive control for typical activation stimuli to assess dynamic range of expression. LPS strongly induced the expression of all surface markers except CD1a.

Given the observed effects with the two representative allergens in these experiments and the reported induction of several markers on Mo-DCs and CD34-DCs (Staquet et al., 2004; De Smedt et al., 2005), we further wanted to confirm the sensitivity and specificity of the CD86, CD54, CD40, and CD83 on THP-1 cells following exposure to a few other allergens and two non-allergens.

### 3.2. Cytokine production induced by DNCB, NiSO<sub>4</sub> and SLS

By recovering the culture supernatant from cells treated with DNCB, NiSO<sub>4</sub> and SLS, we also evaluated the production of TNF-α, IL-6 and IL-8 (Fig. 3). DNCB and NiSO<sub>4</sub> statistically significantly increased the production of TNF-α and IL-8 in a dose-dependent manner. IL-6 secretion was detected and statistically significantly increased

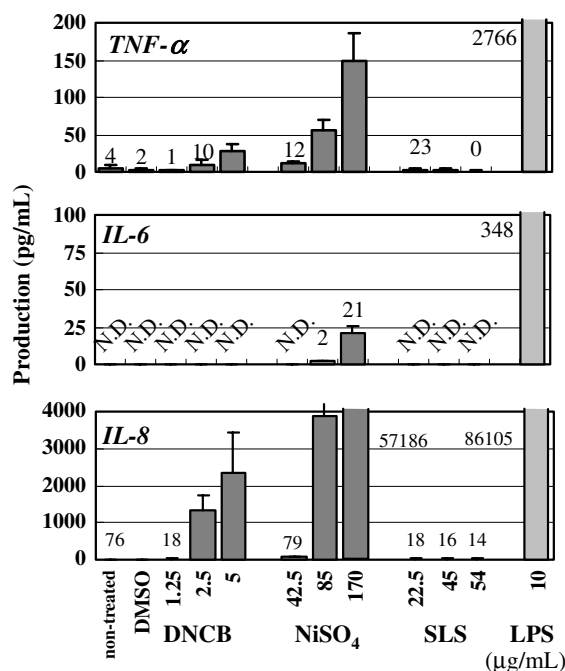


Fig. 3. Changes of cytokine production by DNCB, NiSO<sub>4</sub>, SLS and LPS at the indicated concentrations. The culture supernatant was measured for TNF-α, IL-6 and IL-8 secretion by ELISA. Mean ± S.D. was given for at least three independent experiments. Statistical significance was calculated by Student's *t*-test (\*\* *p* < 0.01; \* *p* < 0.05). N.D.—not detected.

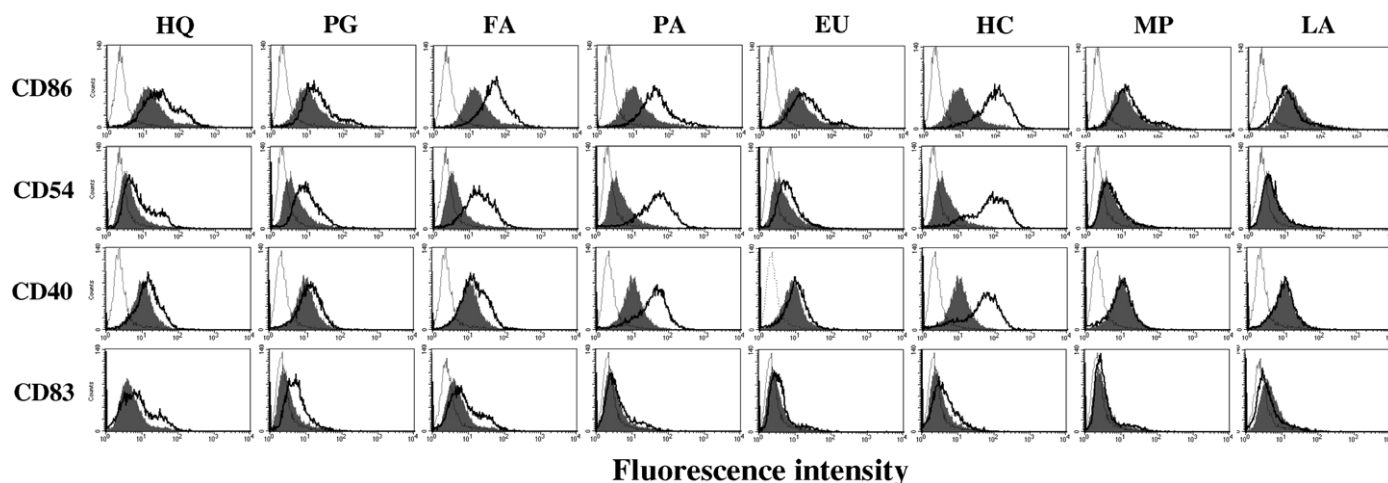


Fig. 4. Effect of six allergens and two non-allergens on surface phenotypic changes. The histograms are representative data at the highest concentration from three independent experiments. Isotype control (dotted line), vehicle-control (shaded peak), and chemical-treated (dark solid line).

only after exposure to  $\text{NiSO}_4$  (170  $\mu\text{g/mL}$ ), in contrast to DNCB and SLS, which showed no effect. Among the three cytokines, levels of IL-8 production by the treatment with DNCB and  $\text{NiSO}_4$  were most significant (e.g. the maximal IL-8 concentration for  $\text{NiSO}_4$  was more than 57,000 pg/mL). SLS did not induce any augmentation of cytokine production at any concentration we examined. LPS, the positive control for activation, was found to be the most potent inducer of production in all tested cytokines.

Cytokine production was also noted to increase with exposure to our two allergen representative (DNCB and  $\text{NiSO}_4$ ). The next set of experiments we did was to evaluate potential changes in cytokine products following exposure to six allergens and two non-allergens in order to confirm the sensitivity and specificity of the cytokines markers (TNF- $\alpha$  and IL-8) on THP-1 cells.

### 3.3. Effect of a panel of chemicals on surface phenotypic changes and cytokine production

To further confirm the sensitivity and specificity of the response of THP-1 cells, we examined the effect of six allergens and two non-allergens on CD86, CD54, CD40 and CD83 expression (Figs. 4, 5a–d, 6, Table 1). Cells were treated for 24 h with three concentrations that caused 70–95% cell viability. Cell viability decreased in a dose-dependent manner with increasing chemical concentration. PAA and HC resulted in a significant increase in all tested surface markers. HQ and FA induced significant up-regulation of CD86, CD40, and CD83 expression in a dose-dependent manner. PG and EU also caused significant increased changes in at least three of tested four surface markers. On the other hand, none of non-allergens, MP and LA, induced any changes in these four phenotypic markers. CD54 expression was strongly induced in a dose-dependent manner for all tested allergens. CD40 expression was also statistically significantly induced for all tested allergens but the

induction was not necessarily dose dependent. The extent of the induction in CD83 expression by allergens is smaller than that in other phenotypic markers while CD83 expression was significantly induced for all tested allergens except EU.

We further examined the effect on TNF- $\alpha$  and IL-8 production (Fig. 5a and b, Table 1). FA and HC significantly induced TNF- $\alpha$  secretion in a dose-dependent manner and resulted in the higher production levels with the highest concentrations compared to the other allergens. PAA also strongly induced TNF- $\alpha$  secretion but the production was not dose-dependent. While HQ induced a slightly significant increase in TNF- $\alpha$  production, PG, EU and non-allergens (i.e., MP and LA) resulted in a low increase in TNF- $\alpha$  secretion without a statistical significant effect. HQ, PAA and HC significantly induced IL-8 secretion in a dose-dependent manner. PG resulted in significant increases at the highest concentration (cell viability = 79%). Although EU and FA did not significantly induce IL-8 production, the dose-dependent production was observed with FA. None of MP and LA concentrations induced any significant changes. Comparing phenotypic alterations, the order of magnitude of cytokine production varied depending on allergen.

## 4. Discussion

In this study, we first investigated phenotypic changes and cytokine production in THP-1 cells when treated with DNCB,  $\text{NiSO}_4$  and SLS. The response was assessed by measuring changes in markers reflecting DC maturation (i.e. CD86, CD54, CD40, CD83, CD58, CD1a, TNF- $\alpha$ , IL-6 and IL-8). Although some of the data presented (up-regulation of cell surface markers CD54 and CD86 on THP-1 by DNCB,  $\text{NiSO}_4$ , and SLS) in this study have been recently reported in Ashikaga et al. (2006) and Sakaguchi et al. (2006), the data are critical for the study. These parameters were repeated in order to provide a good reference and

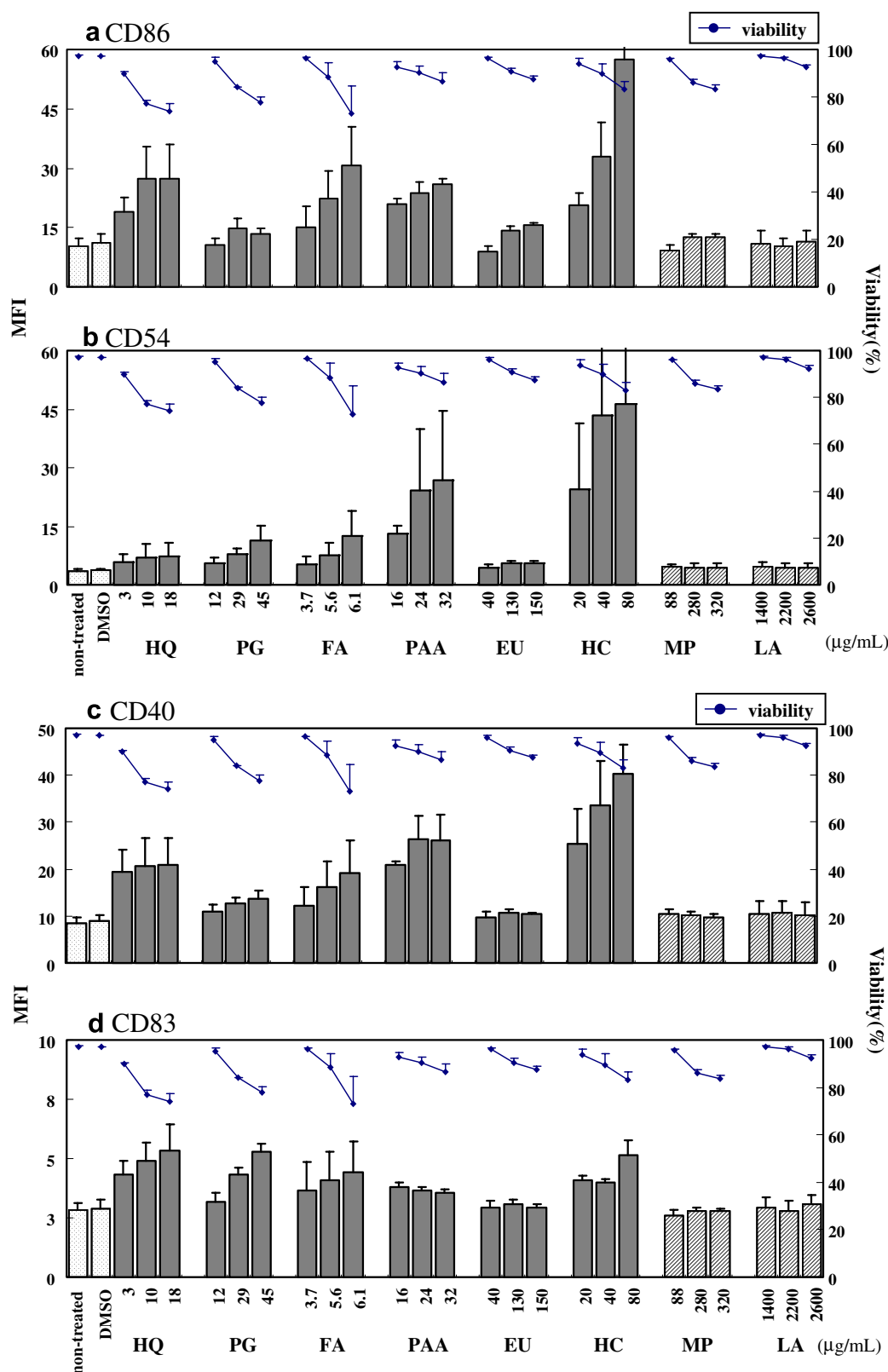


Fig. 5. The effect of six allergens and two non-allergens on expression of (a) CD86, (b) CD54, (c) CD40 and (d) CD83. Cells were treated for 24 h at the indicated concentration. Mean  $\pm$  S.D. was given for at least three independent experiments. Statistical significance is reported in Table 1.

control for this study as well as validate the results of the previous study. Both DNCB and NiSO<sub>4</sub> are representative allergens and have been reported to induce several markers

on Mo-DCs and CD34-DCs. As observed in our first set of experiments, CD86, CD54, CD40, CD83, TNF- $\alpha$  and IL-8 on THP-1 cells were markers that were the most reactive to

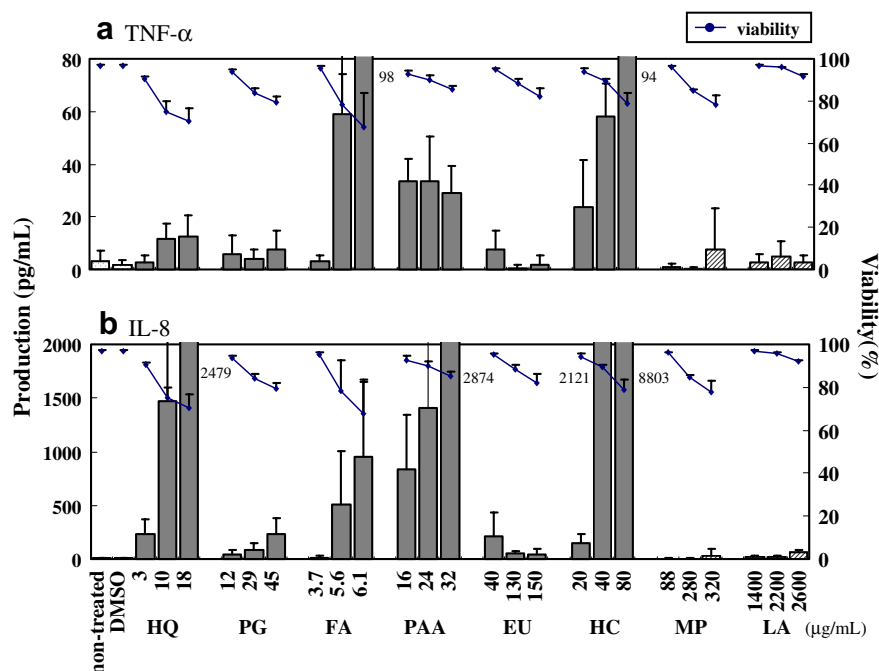


Fig. 6. The effect of six allergens and two non-allergens on production of (a) TNF- $\alpha$  and (b) IL-8. Cells were treated for 24 h at the indicated concentration. Mean  $\pm$  S.D. was given for at least three independent experiments. Statistical significance is reported in Table 1.

DNCB and NiSO<sub>4</sub>. As such, we further wanted to confirm the sensitivity and specificity of these markers by examining the effect of six allergens and two non-allergens.

We found that non-treated THP-1 cells were CD58<sup>+</sup>, CD86<sup>low</sup> and CD40<sup>low</sup>, but were nearly negative for CD54, CD83 and CD1a. As for the data results, we can only say it is the characteristic of THP-1 cells that CD86 and CD40 basal expression are higher than those of CD54 and CD83. In addition, THP-1 cells augmented expression of CD86, CD54, CD40, CD83 and production of TNF- $\alpha$  and IL-8 in response to various allergens, but did not in response to non-allergens. These alterations are consistent with the response observed by Mo-DCs and CD34-DCs (Aiba et al., 1997, 2003; Coutant et al., 1999; De Smedt et al., 2001, 2002; Staquet et al., 2004).

CD40, a member of the tumor necrosis factor receptor family, is expressed on B cells, macrophages and DCs (Van Kooten and Banchereau, 1997; Pinchuk et al., 1996). CD40-CD40 ligand interactions induced up-regulation of CD54 and CD86 expression on human cultured LCs and resulted in enhanced allostimulating capacity (Peguet-Navarro et al., 1995). Thus, CD40 might be involved in the DC-induced T cell response. In this study, all tested allergens significantly induced CD40 expression on THP-1 cells, but the non-allergens did not. NiSO<sub>4</sub> and HC induced a stronger up-regulation in a dose-dependent manner in the cell viability range from 79% to 94% and increased CD40 expression over three times at the highest concentration, compared to control cells. HC, considered as a weak allergen in LLNA (Basketter et al., 1994), strongly activated THP-1 cells for all tested markers. To our knowledge, the effect of HC on Mo-DCs or CD34-DCs has been not

reported, but THP-1 cell activation induced by HC might be involved in a different signaling pathway from that of the other allergens. Similar to our results, the up-regulation of CD40 expression induced by several allergens including DNCB and NiSO<sub>4</sub> has been also confirmed in Mo-DCs (Coutant et al., 1999; Staquet et al., 2004). These data suggested that THP-1 cells could induce the up-regulation of CD40 expression by allergens.

CD83 is known to be DC lineage specific marker. Although the function of CD83 is still unknown in the induction phase of skin sensitization, the pattern of expression of this molecule suggests that it may serve a pivotal role in antigen presentation or cellular interaction (Zhou and Tedder, 1995). In THP-1 cells, CD83 expression was significantly induced after exposure to all tested allergens except EU. Depending on the allergen, the dose-dependent up-regulation in CD83 expression was observed in less time than other phenotypic markers. When cells were exposed to DNCB, CD83 expression was significantly augmented at the middle dose. De Smedt et al. (2005) reported that DNCB and EU failed to induce CD83 expression according to analysis of the mean fluorescence intensities when CD34-DCs were treated at the similar concentration for 24 h. It is suggested that CD83 expression in THP-1 cells could exhibit a higher sensitivity to various allergens than that found in CD34-DCs.

TNF- $\alpha$  plays important roles in the maturation and migration of LCs (Wang et al., 1999; Salgado et al., 1999). Flint et al. (1998) have shown that topical exposure of mice to oxazolone, a known allergen, induced cutaneous TNF- $\alpha$  production, which was tightly controlled in space and time. In our study, a significant production of this cytokine was

observed when THP-1 cells were exposed to allergens except PG and EU. The magnitude of cytokine production varied depending on allergen and was also likely independent of the classification of allergens using LLNA. On the other hand, Manome and Aiba (1999) observed that only  $\text{NiCl}_2$  and  $\text{MnCl}_2$  induced the significant production of  $\text{TNF-}\alpha$  in Mo-DCs among 14 chemicals tested including DNCB. The up-regulation of  $\text{TNF-}\alpha$  induced by various allergens in THP-1 cells, which is more sensitive than Mo-DCs, might be due to unique features of the THP-1 cells.

The chemokine IL-8 is known to be involved in the inflammation reaction and its role in skin sensitization process is currently being investigated. IL-8 production in THP-1 cells was up-regulated by almost all allergens tested, and the extent of increase was allergen-dependent and found to be similar to that of  $\text{TNF-}\alpha$ . Some investigations have recently reported that DNCB and  $\text{NiCl}_2$  induced a strong up-regulation in IL-8 production in Mo-DCs (Aiba et al., 2003); and that DNCB and  $\text{NiSO}_4$ , not EU, induced the significant up-regulation in IL-8 mRNA level in CD34-DCs (Verheyen et al., 2005). Although two cytokines (i.e.,  $\text{TNF-}\alpha$  and IL-8) in our study could be released in response to various allergens, questions still remain about the variation of production between allergens and the effect of released cytokines on cell activation (e.g., autocrine signaling).

CD1a protein on LCs has been shown to bind and present lipid antigens to T cells, similar to MHC class II (Salamero et al., 2001). During the maturation, LCs decrease CD1a expression. In THP-1 cells, the expression of CD1a was augmented following exposure to DNCB at the middle dose, but  $\text{NiSO}_4$  and even LPS, a positive control for activation, did not induce CD1a expression. When Mo-DCs were exposed to DNCB and  $\text{NiSO}_4$ , significant changes of CD1a expression have not been previously observed (Aiba et al., 1997). The effect of allergens to CD1a expression in THP-1 cells remains to be investigated.

IL-6 has been shown to be required for optimal T cell activation (Hope et al., 2000). In our study, IL-6 production was significantly augmented following treatment with  $\text{NiSO}_4$  (170  $\mu\text{g/mL}$ ) but not detected with DNCB treatment at three concentrations. Aiba et al. (1997) reported that  $\text{NiCl}_2$ , but not DNCB up-regulated the production of IL-6 in Mo-DCs. Recently, the increased change in IL-6 mRNA levels was observed on CD34-DCs treated with  $\text{NiSO}_4$ , but not DNCB (Verheyen et al., 2005). On the other hand, De Smedt et al. (2005) found DNCB and  $\text{NiSO}_4$  to induce an increase of IL-6 production in CD34-DCs, but noticed that the sensitivity of this cytokine was lower compared to that of surface markers (e.g., CD86). These data suggest that the response of IL-6 to allergens might be similar but low in three cell types.

The high expression of CD58 molecules on LC has been shown to considerably contribute to powerful antigen presentation function and T cell activation (Teunissen et al., 1994). In THP-1 cells, no significant changes in CD58 expression were observed with DNCB and  $\text{NiSO}_4$  treat-

ment. This might attribute to the inherent high expression levels of CD58 on THP-1 cells compared to other surface markers.

We also found that  $\text{NiSO}_4$  was more efficient in CD54 and CD40 expression and secretion of  $\text{TNF-}\alpha$ , and IL-8 than DNCB. For example, the extent of up-regulation between DNCB and  $\text{NiSO}_4$  is about two fold different in these markers when comparing similar cytotoxic DNCB (5  $\mu\text{g/mL}$ ) and  $\text{NiSO}_4$  (85  $\mu\text{g/mL}$ ) concentrations. DNCB and  $\text{NiSO}_4$  induced CD54 and CD40 expression in a dose-dependent manner, but did not change CD86 and CD83 expression. Other tested chemicals also had a similar pattern in that one surface marker may be induced dose-dependently while another marker may not. These results revealed that the concentration does not necessarily correlate to the ability of the chemicals to induce markers on THP-1 cells. Mizuashi et al. (2005) reported that DNCB may reduce GSH/GSSG ratio in Mo-DCs without generating reactive oxygen species (ROS), which is accompanied by the phosphorylation of p38 MAPK and a subsequent augmentation of CD86 expression, whereas  $\text{NiCl}_2$  may decrease the GSH/GSSG ratio by producing ROS. Therefore, we speculate that different intracellular signaling pathways might exist in THP-1 cells and result in the difference of THP-1 cell activation between DNCB and  $\text{NiSO}_4$ .

In this study, the expression of functionally distinct surface markers and cytokines was activated on THP-1 cells in response to several allergens. Furthermore, the observed allergen-induced activation apparently is associated with slight cytotoxicity (70–95% cell viability). On the other hand, THP-1 cell activation was not induced at slight cytotoxic concentrations of non-allergens. This is consistent with the results of Hulette et al. (2005). They reported that up-regulation of CD86 expression in Mo-DCs was observed when cells were exposed to allergens at concentrations which induced 10–15% cytotoxicity. Therefore, in THP-1 cells and Mo-DCs, the close association between cell activation and cytotoxicity might exist and be allergen specific. Manome and Aiba (1999) examined the mode of death of Mo-DCs when exposed to some allergens, including DNCB and  $\text{NiCl}_2$ . Their observation revealed that  $\text{NiCl}_2$  induced significant numbers of apoptotic cells at 1 mM concentration or more, while DNCB induced necrotic cells at any concentration they examined. It was suggested that the mode of death induced by both allergens and irritants are not necessarily uniform and are dependent on chemicals. Further work is needed to address this aspect in THP-1 cells.

In this study, the mechanistic link to T cell presentation does not become clear and is an important issue to be resolved. It is well reported that allergen responses, in the form of CD86 and CD83 expression, have been observed in Mo-DCs. Our study revealed that THP-1 cells can also modulate several markers, which are related to DC maturation, following treatment with allergens. Judging from these common phenomena, we believe that THP-1 cell activation may have a role in T cell presentation.

In conclusion, THP-1 cells have the high capacity to change the markers reflecting DC maturation and then to distinguish between allergens and non-allergens in the similar manner as those reported for DCs (e.g. Mo-DCs, CD34-DCs). In contrast to large donor to donor variability in Mo-DCs reported by some investigators (Aiba et al., 1997; Tuschl et al., 2000; Staquet et al., 2004), THP-1 cells have the advantage of being more homogeneous and reproducible. Therefore, THP-1 cells, which develop several DC-like properties, can be used in a suitable model capable of identifying sensitizing potential of chemicals.

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