

# MOLECULAR EVENTS ASSOCIATED WITH DENDRITIC CELLS ACTIVATION BY CONTACT SENSITIZERS

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**Abstract.** Dendritic cells are unique heterogenous cell population capable of responding to different inflammatory signals. These signals induce the process of maturation forcing cells to leave the inflamed site and to migrate to draining lymph nodes. During this process, dendritic cells have to initiate differentiation responses expressed among others by up-regulation of some surface antigens (e.g., MHC or costimulatory molecules) and down-regulation of the others (e.g., E-cadherin). There are more and more data published indicating that chemical compounds known to initiate contact hypersensitivity (haptens) can also induce the process of dendritic cells maturation resembling in some aspects their maturation after contact with common inflammatory factors. Although molecular events associated with the process have not yet been clearly defined, there is growing evidence that haptens mediate activation of dendritic cells by activating mitogen-activated protein kinases, mainly p38 MAPK. It may be speculated that further downstream signals include activation of transcription factors such as nuclear factor  $\kappa$ -B (NF- $\kappa$ B), activating transcription factor-2 (ATF-2) or cAMP response element-binding protein (CREB). The present review will focus on some aspects of signal transduction pathways in dendritic cells stimulated with contact sensitizers.

**Key words:**

Dendritic cells, Langerhans cells, Contact sensitizers, Signal transduction

## INTRODUCTION

Contact dermatitis characterized by inflammatory reaction of the skin resulting from exposure to exogenous substances can be elicited by two mechanisms: irritant or allergic. Irritant contact dermatitis (about 80% of cases of contact dermatitis) is caused by direct damaging effect of a xenobiotic to the skin. Allergic contact dermatitis (also known as “type IV” or “delayed hypersensitivity” reaction) accounting for the remaining 20% of cases is a body’s immune system reaction to a particular substance or group of related substances. The most common allergens include: metals (nickel, chromium), fragrances, rubber, some plants, formaldehyde, skin medications, hairdressing

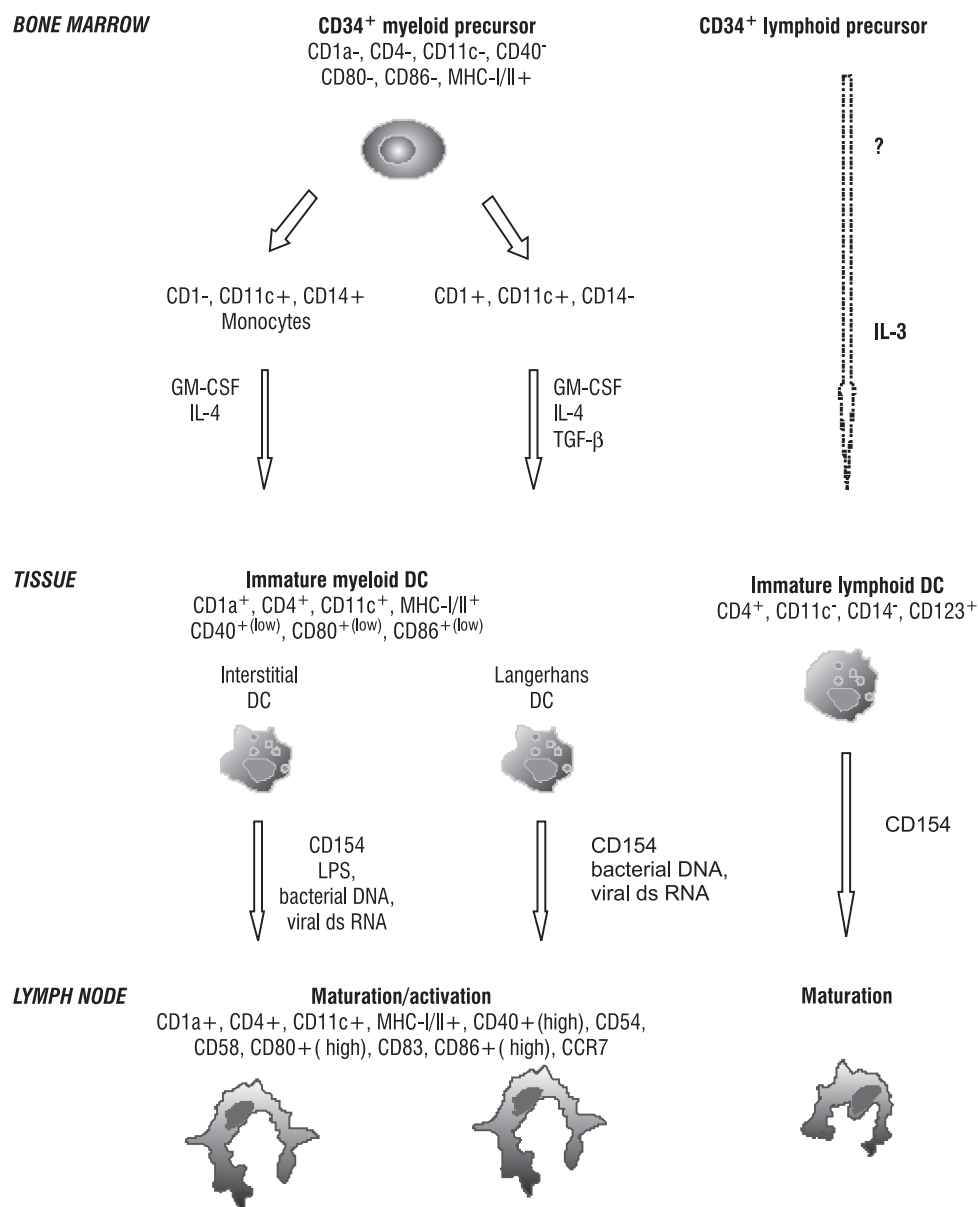
chemicals. Allergic contact dermatitis is characterized by a delay between first exposure to an allergen (sensitization phase) and the subsequent reaction (elicitation phase).

It is suggested that an important role in the sensitization phase of allergic contact dermatitis is played by dendritic cells (DCs) constituting a family of antigen-presenting cells (APC) which capture different allergens/pathogens for processing and presentation to T cells in the secondary lymphoid organs. DCs can be found in different anatomical areas, e.g., epidermis (Langerhans cells, LCs), dermis (interstitial or dermal DCs), spleen, T zones (interdigitating DCs) and germinal centers of lymphoid organs, thymus, liver, and blood.

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**Fig. 1.** Scheme of DCs differentiation as well as phenotypic and functional changes linked to their maturation/activation (for details see text (4)).

In humans, there are generally three subpopulations of DCs: two originating from the myeloid lineage, interstitial DCs and Langerhans cells (LCs), and the third, the lymphoid DCs. Recent developments in the field of *in vitro* cultures have permitted generation of huge numbers of highly purified DCs derived from the CD34<sup>+</sup> myeloid or lymphoid blood precursors. Researchers are able to culture immature interstitial DCs from monocyte CD1<sup>-</sup>, CD11c<sup>+</sup>, CD14<sup>+</sup> precursors using granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) [1]. Myeloid precursors can also differentiate into CD1<sup>+</sup>,

CD11c<sup>+</sup>, CD14<sup>-</sup> precursors which, upon stimulation with GM-CSF and IL-4 and transforming growth factor- $\beta$  (TGF- $\beta$ ), can yield Langerhans cells [2]. Lymphoid DCs are CD4<sup>+</sup>, CD11c<sup>-</sup>, CD14<sup>-</sup>, and IL-3 $\alpha$ <sup>+</sup>/CD123<sup>+</sup>. They rapidly die after isolation, requiring IL-3 for survival and CD40L/CD154 for maturation [3].

The encounter of an antigen or pathogen evokes phenotypic and functional changes in the immature DCs which are associated with their transition from antigen-capturing and processing cells to antigen-presenting cells. This process of DCs maturation starting at the moment of antigen/

pathogen binding induces migration of the cells from the peripheral tissue (e.g., skin) to the draining lymphoid organs. Actually, maturation of DCs is a continuous process that terminates upon their contact with and stimulation of T lymphocytes.

Maturation of DCs in *in vivo* and *in vitro* systems can be induced by different stimuli like immunoreactive molecules (tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), GM-CSF, T-cell CD40L/CD154) and bacterial/viral products (lipopolysaccharide (LPS), lipoteichoic acid, lipoarabinomannan, CpG motifs in bacterial DNA, double-stranded viral RNA) [4]. Phenotypic and functional changes linked to DCs maturation consist of loosing endocytic and phagocytic receptors and up-regulation of levels of surface molecules involved in antigen presentation and T-cell activation (shift from antigen capture and processing to antigen presentation) [5] (Fig. 1).

Up-regulated molecules include, among other things:

- the major histocompatibility complex, class II (MHC II) molecules (increased synthesis and translocation of the MHC II-peptide complexes, exhibiting prolonged half-life to the cell surface, facilitate their recognition by CD4<sup>+</sup> T cells),
- accessory/costimulatory molecules required for lymphocyte activation like: intercellular adhesion molecule (ICAM)-1/CD54, leukocyte functional antigen-3 (LFA)-3/CD58, B7-1/CD80, B7-2/CD86, and CD83-unique marker of matured DCs,
- CD40 important in T cells interactions; DCs can be activated by T cells via this receptor, which leads to increased expression of CD80/CD86 and cytokine release (IL-1, TNF, chemokines, and IL-12),
- chemokine receptor-7 (CCR7); the receptor plays a role in chemotaxis of DCs to secondary lymphoid organs in response to 6Ckine and macrophage inflammatory protein-3b (MIP-3b), which are produced primarily in the T cell-rich parafollicular areas of lymphoid tissues [6].

A growing number of recently published papers points to strong contact sensitizers regarded as factors inducing maturation/activation of different subpopulations of DCs. Similarities between molecular events (e.g., expression of surface antigens) observed in DCs induced with typical

stimulators and sensitizers allow to assume the existence of some common mechanisms of DCs activation in both cases. Although, more and more is known about early events of DCs activation following the exposure to sensitizers, little is known about later stages, e.g., activation of transcription factors or transcription of genes.

### CHANGES IN EXPRESSION OF CELL MEMBRANE ANTIGENS IN DCS FOLLOWING EXPOSURE TO CONTACT SENSITIZERS

Putative mechanisms responsible for reactions of contact allergens with cell amino (lysine residues) and thiol (cysteine residues) groups have been proposed as suspected of involvement in hypersensitivity induction [7]. Becker et al. [8] documented the ability of 2 contact allergens, 5-chloro-2-methylisothiazolinone plus 2-methylisothiazolinone (MCI/MI) and 2,4,6-trinitrochlorobenzene (TNCB), as well as the thiol-group-specific reagents, N-hydroxymaleimide (NHM), and N-ethylmaleimide (NEM) to strongly induce tyrosine phosphorylation in CD14<sup>+</sup> monocytes isolated from human peripheral blood. In the contrary, sulfosuccinimidyl acetate (sulfo-NHS) and 2-iminothiolane, common amino-group-specific reagents, did not induce tyrosine phosphorylation. Moreover, cysteine (but not lysine) present in incubation medium blocked tyrosine phosphorylation, which could suggest a competitive inhibition of free thiol groups, but not amino groups, of activation of tyrosine kinases by MCI/MI and TNCB. Contact sensitizers are able to up- ( $\uparrow$ ) or down-regulate ( $\downarrow$ ) the expression of different molecules on dendritic cells (DCs) which are involved in their maturation and activation (Table 1).

It seems that exposure of DCs to haptens induces early transient internalization of MHC class II molecules followed by their increased expression later on. In mice, after 3h of *in vivo* application of 2,4-dinitrofluorobenzene (DNFB), a decrease in the Ia-antigen expression on the entire LC population was noted. Similar phenomenon was observed after *in vivo* application of 1-chloro-2,4-dinitrobenzene (DNCB), oxazolone, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, 2,4,6-trinitrochlorobenzene as well as toxic concentrations of sodium



**Table 1.** Surface antigen changes observed in different subpopulations of dendritic cells exposed to contact sensitizers

| Surface antigen               | DCs subpopulation                                                                                                                    | Reference                 |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| ↑ MHC class II                | Mouse LCs                                                                                                                            | 9–11                      |
| ↑ MHC class II                | Mouse bone marrow-derived XS52 cell line                                                                                             | 12                        |
| ↑ HLA-DR                      | Human monocyte-derived DCs                                                                                                           | 13–16                     |
| ↑ CD23                        | Mouse LCs                                                                                                                            | 17                        |
| ↑ CD32/16                     | Mouse LCs                                                                                                                            | 17                        |
| ↑ CD40                        | Mouse LCs                                                                                                                            | 10                        |
| ↑ pan CD44                    | LCs and DCs                                                                                                                          | 18, 19                    |
| ↑ CD44v4,v5,v6,v9             | LCs and DCs                                                                                                                          | 18, 19                    |
| ↑ CD45                        | Mouse LCs                                                                                                                            | 17                        |
| ↑ CD54                        | Mouse LCs, human monocyte-derived DCs                                                                                                | 10, 13, 15, 19            |
| ↑ CD80                        | Mouse LCs                                                                                                                            | 10                        |
| ↑ CD83                        | Langerhans-like DCs derived from human cord blood progenitors                                                                        | 14                        |
| ↑ CD86                        | Mouse LCs, human monocyte-derived DCs, Langerhans-like DCs derived from human cord blood progenitors, THP-1 human monocyte cell line | 10, 13, 14, 20 15, 16, 19 |
| ↑ Type IV collagenase (MMP-9) | Mouse LCs                                                                                                                            | 21                        |
| ↑ Chemokine receptor 7 (CCR7) | TGF- $\beta$ 1 <sup>+</sup> DCs                                                                                                      | 19                        |
| ↑ $\alpha_5\beta_1$ -integrin | TGF- $\beta$ 1 <sup>+</sup> DCs                                                                                                      | 19                        |
| ↓ E-cadherin                  | Mouse LCs, human LCs                                                                                                                 | 11, 22                    |
| ↓ c-fms DCs                   | Human monocyte-derived                                                                                                               | 16                        |

dodecyl sulfate (SDS) [23]. Similarly, Ashikaga et al. [20] observed internalization of MHC class II molecules on human monocyte cell line THP-1 pretreated with interferon- $\alpha$  (IFN- $\alpha$ ) for 24 h and then stimulated for 2 h with strong sensitizers (DNCB, 2-mercaptobenzothiazole, p-phenylenediamine, ammonium tetrachloroplatinate) but not with irritants (SDS, methylsalicylate or DMSO).

Increased expression of class II MHC I-A $\alpha$  on mouse LCs after hapten application (DNCB, TNCB, DNFB) was ascribed to interleukin-1 (IL-1) produced by LCs themselves [9]. Langerhans cells were the primary source of the IL-1 $\beta$ . In the study, antigen I-A $\alpha$  as well as IL-1 $\alpha$ , IL-1 $\beta$ , IFN-induced protein 10 (IP-10), and macrophage inflammatory protein 2 (MIP-2) mRNAs were up-regulated in the epidermis only after allergen but not after irritant or tolerogen painting. Herouet et al. [12] also demonstrated increased expression of MHC class II molecules observed within 18 h on the XS52 mouse bone marrow-derived cell line after stimulation with dinitrobenzene sulfonic acid (DNBS) or oxazolone.

Ozawa et al. [10] observed that murine Langerhans cells cultured and activated *in vitro* by haptens and the cells activated *in vivo* by hapten painting showed increased expression of Ia antigen and the co-stimulatory molecules: CD40, CD54 (ICAM-1), CD80, and CD86. Additional surface

molecules up-regulated after *in vitro* treatment of mouse LCs with trinitrobenzene sulfonic acid (TNBS) were Fc $\epsilon$ RII (CD23), Fc $\gamma$ RII/III (CD32/CD16) and CD45 [17]. Human monocyte-derived CD1a<sup>+</sup> DC exposed *in vitro* to DNCB and NiCl<sub>2</sub>, but not to ZnCl<sub>2</sub>, sodium dodecyl sulfate, or benzalkonium chloride (BC) significantly increased the surface expression of CD54, CD86, HLA-DR antigen, and IL-1 $\beta$  production (only SDS weakly augmented CD86 expression) [15]. The up-regulation of CD86 expression on DC treated with DNCB was significantly suppressed by anti-IL-1 $\beta$  and anti-TNF- $\alpha$  antibodies. The effects of NiCl<sub>2</sub> were rather insensitive to these antibodies, suggesting different pathways of DCs activation by the two chemicals. The involvement of protein kinase C in activation of DCs was shown using a specific inhibitor H7 (1-(5-isoquinolinesulphonyl)-2-methylpiperazine).

The Langerhans-like dendritic cells derived from human cord blood progenitors in response to strong haptens like TNBS, fluorescein isothiocyanate or Bandrowski's base (N',N'-bis(4-aminophenyl)-2,5-diamino-1,4-quinone-diimine), but not to weak allergens or irritants (SDS), stimulated proliferation of autologous T lymphocytes [16]. The haptens also effectively induced maturation of DCs as judged by increased expression of CD83, CD86 and HLA-DR molecules.

Additional data on human monocyte-derived DCs stimulated with contact sensitizers were provided by Tuschl et al. [15]. The authors were able to observe up-regulation of CD54, CD86, and HLA-DR molecules on DCs in response to NiSO<sub>4</sub>, DNCB, TNBS,  $\alpha$ -hexylcinnamaldehyde and eugenol. Sodium dodecyl sulfate and the vehicle dimethyl sulfoxide (DMSO) had no effect.

Interesting effect of haptens on E-cadherin expression (cell adhesion molecule belonging to a group of developmental proteins responsible for maintaining the structural integrity of epithelial monolayers and DC interactions with keratinocytes in the skin) was observed by Schwarzenberger et al. [11]. TNCB was shown to increase I-A/E antigen and decrease E-cadherin expression on exposed *in situ* murine LCs as early as 12 h, and as late as 48 h after application. No such changes could be observed in LCs after treatment with several contact irritants or the tolerogen dinitrothiocyanobenzene. Interestingly, IL-1 and TNF- $\alpha$  injected into ear skin similarly decreased E-cadherin expression. Verrier et al. [22] also observed down-regulation of E-cadherin expression on human epidermal LCs as well as a significant decrease in the percentage of E-cadherin-positive cells as a result of exposure to contact sensitizers isoeugenol, cinnamaldehyde, TNBS, Bandrowski's base or p-phenylenediamine, but not to SLS. These observations suggest that exposure of LCs to contact sensitizers and thereby down-regulation of E-cadherin expression might facilitate the release of LCs from the epidermis and initiate their migration to local lymph nodes. During this journey, LCs have to transmigrate through the basement membranes of different structures. It may be speculated that matrix metalloproteinases (MMPs), up-regulated in LCs, can play a significant role in this process. Indeed, type IV collagenase (MMP-9) was found to be produced in LC-enriched epidermal cells isolated 6 h after application of TNCB [21].

MMP-9 expression substantially increased between 12 and 24 h, and then returned to normal levels by 7 to 10 days. MMP-9 expression could also be observed after application of DNCB and DNFB. Further support of the involvement of MMPs in transmigration of activated LCs lent observations of Lebre et al. [24] who demonstrated that

in the presence of the MMPs inhibitors (BB94, BB2116 and CT1166), NiSO<sub>4</sub>-induced migration of LC from the cultured skin explants was strongly decreased.

Another interesting point in the mechanism of the initiation of LCs migration after their contact with haptens was provided by Weiss et al. [18]. They showed that stimulated LCs and DCs up-regulated pan CD44 epitopes and epitopes encoded by variant exons v4, v5, v6, and v9. Moreover, antibodies against CD44 epitopes inhibited the emigration of LCs from the epidermis and binding of activated LCs and DCs to the T cell zones of lymph nodes.

There are data indicating that DCs in response to haptens (NiCl<sub>2</sub> and DNCB) increase expression of mRNA for chemokine receptor 7 (CCR7) [19]. This suggests an existence of another facilitating mechanism of emigration of DCs from peripheral tissue (e.g., skin) to lymph nodes via lymphatics.

It seems, however, that phenotypic changes of DCs induced by various haptens may be different and characteristic of particular hapten. Aiba et al. [19] showed that the TGF- $\beta$ 1<sup>+</sup> DCs stimulated with NiCl<sub>2</sub>, but not with DNCB, exhibited increase in expression of CD54, HLA-DR,  $\alpha_5\beta_1$ -integrin, CD44 and CD44v6. Moreover, DNCB seemed to stimulate, at first, TGF- $\beta$ 1<sup>+</sup> DCs to secrete IL-1 $\beta$  or TNF- $\alpha$ , which in consequence induced their CD86 expression. On the other hand, NiCl<sub>2</sub> directly induced the augmentation of CD86 expression on TGF- $\beta$ 1<sup>+</sup> DCs.

Similar differences in potential of haptens for induction of co-stimulatory molecules expression on DCs was demonstrated by Ashikaga et al. [20] who observed that in THP-1 human monocyte cell line DNCB, 2-mercaptobenzothiazole, eugenol, p-phenylenediamine and ammonium tetrachloroplatinate increased CD86 expression, while NiSO<sub>4</sub>, CoSO<sub>4</sub>, and irritants such as methylsalicylate, SDS and DMSO did not.

Manome et al. [16] demonstrated that increased expression of CD86 and HLA-DR molecules on human monocyte-derived DCs was not necessarily a specific feature of sensitizers. The authors noticed such effects after stimulation of the cells with selected metal salts, i.e., MnCl<sub>2</sub>, SnCl<sub>2</sub>, CdSO<sub>4</sub>. In addition, down-regulation of c-fms (encoding the colony-stimulating factor CSF-1 receptor)



in DCs was observed not only for strong sensitizers like DNCB, DNFB,  $\text{NiCl}_2$ , but also for  $\text{MnCl}_2$ .

#### EVIDENCE FOR THE ACTIVATION OF CELLULAR TYROSINE KINASES FOLLOWING EXPOSURE OF DCs TO CONTACT SENSITIZERS

There are several recent reports indicating an increase in the amount of phosphotyrosine (p-tyr) and the activation of mitogen-activated protein kinases (MAPKs) in MHC class II cells stimulated with strong sensitizers.

The mitogen-activated protein kinases (MAPKs) constitute a family of kinases including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal protein kinase or stress activated protein kinase (JNK/SAPK), and p38 kinase. The ERKs are the prototypic MAPKs and are activated by a variety of mitogenic stimuli as well as by differentiation signals, whereas p38 and JNK are usually referred to as stress-stimulated MAPKs, which mediate the induction of apoptosis by diverse stimuli such as UV irradiation, osmotic shock, genotoxic agents, pro-inflammatory cytokines and oxidants.

Kuhn et al. [25] were able to observe increased tyrosine phosphorylation after stimulation with MCI/MI in human LCs isolated from the skin and cultured for 24 h as well as in B lymphocytes, fresh DCs (f-DCs) and cultured DCs (c-DCs) isolated from the blood. This process was quite rapid - p-tyr generation in f-DCs was discernible after 4 min of incubation and maximal after 15 min. Kinase involvement was confirmed using their specific inhibitors: tyrphostin B56, herbimycin A and genistein. Western blot analysis of tyrosine-phosphorylated proteins in lysates from monocyte-enriched populations after stimulation with MCI/MI for 15 min showed that neither p38 MAP-kinase nor p85 PI3 kinase were the targets for kinases induced after hapten stimulation. Analysis of c-DCs exposed to DNFB showed increased phosphorylation of proteins of about 41, 65, and 75 kDa. Interestingly, the proteins of 65 and 75 kDa resembled proteins being hyperphosphorylated in monocyte-enriched cells after cross-linking with anti-HLA-DR antibodies, which might suggest an as yet unidentified common mechanism of activation in both cases.

DNFB-induced phosphorylation of 65 and 75 kDa proteins was blocked with genistein. Moreover, this inhibitor was also able to block production of mRNA for IL-1 $\beta$ .

More interesting insights into kinase involvement in activation of DCs by haptens were provided by Arrighi et al. [26]. They found that  $\text{NiSO}_4$  and DNFB, but not irritants such as SDS and benzalkonium chloride (BC), induced p38 MAPK phosphorylation in immature monocyte-derived DCs (in contrast to the lack of stimulation observed by Kuhn et al. [25]). Again, the process was quite rapid - detectable increase in phosphorylation was noticed after 5 min and continued during 1 h of incubation. Western blot analysis showed a slight increase in ERK1/2 phosphorylation after  $\text{NiSO}_4$ , but not after DNFB stimulation. No p46/54 JNK phosphorylation could be observed following neither hapten nor irritant stimulation. Additionally, it was shown that up-regulation of CD80, CD83, and to a lesser extent of CD86 molecules on DCs after stimulation with  $\text{NiSO}_4$  was blocked with the p38 MAPK inhibitor SB203580. No effects of the inhibitor were observed on up-regulated CD1a, CD40, and HLA-DR molecules.

Confirmation of an important role of p38 MAPK in the activation of monocytes derived from the human blood by strong sensitizers was provided by Brand et al. [27]. As analyzed by FACS, the cells incubated with MCI/MI, thimerosal, formaldehyde, and DNFB showed increase in tyrosine phosphorylation, while those incubated with BC and  $\text{H}_2\text{O}_2$  (the inductor of oxidative stress) failed to do so. Although, p38 kinase was phosphorylated in monocytes stimulated with haptens and also with SDS and  $\text{H}_2\text{O}_2$ , only haptens were able to induce the translocation of p38 in the active form from the cytoplasm to the detergent-resistant cell fraction. Moreover, only sensitizers induced rapid phosphorylation of p38 MAPK-dependent transcription factor Elk-1, as well as IL-1 $\beta$  release from monocytes.

#### ACTIVATION OF TRANSCRIPTION FACTORS FOLLOWING EXPOSURE OF DCs TO CONTACT SENSITIZERS

In the light of the available evidence, it might be assumed that contact sensitizers can activate different signaling

pathways leading to activation of different transcription factors. The data quoted above suggest the most probable pathway being activated by the p38 subfamily of MAPKs, consisting of p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$ , and p38 $\delta$  proteins, among which targets are:

a) **proteins:**

- MAP kinase-activated protein kinase 2/3 (MAPKAP kinase-2/3), and other kinases Mnk 1 and 2, and Msk-1; MAPKAP kinase 2 and Msk-1 in turn activate the transcription factors – activating transcription factor-1 (ATF-1) and c-AMP responsive element binding protein (CREB),

- p38 regulated/activated protein kinase (PRAK) phosphorylating a small heat shock protein 27 (hsp27) [28];

b) **transcription factors** inducing the expression of many different genes, including proinflammatory cytokine genes:

- Nuclear factor  $\kappa$ -B (NF- $\kappa$ B),

- Elk1 (also known as p62 ternary complex factor, TCF) – an Ets-related transcription factor binding to DNA in part via interaction with serum response factor [29],

- CCAAT/enhancer-binding protein-homologous protein (CHOP; also known as GADD153),

- myocyte enhancer binding factor 2A and 2C (MEF2A, MEF2C),

- activating transcription factor-2 (ATF-2).

All p38 proteins are activated through phosphorylation on threonine and tyrosine residues by a dual-specificity kinase, MAPK kinase 6 (MKK-6); p38 $\alpha$ , p38 $\gamma$ , and p38 $\delta$  are also activated by MAPK kinase 3 (MKK3). Activity of p38 $\alpha$  and p38 $\beta$  can be specifically blocked by the pyridinyl imidazole compound SB203580 binding in the ATP pocket (p38 $\gamma$  and p38 $\delta$  are not sensitive to this compound).

There are available data suggesting that common metal haptens, NiCl<sub>2</sub>, and CoCl<sub>2</sub>, induce the formation of reactive oxygen species that activate NF- $\kappa$ B [30,31]. Also Goebeler et al. [32] reported that NiCl<sub>2</sub> and CoCl<sub>2</sub> induced transcription of mRNA for ICAM-1, VCAM-1 and E-selectin in endothelial cells via NF- $\kappa$ B-dependent signal transduction. Taking into account, for example the known capacity of haptens to release IL-1 $\beta$  from DCs [13,33], one may notice some similarities between activa-

tion of these cells by haptens and LPS. In support of such a theory, Rescigno et al. [34] reported that LPS induced nuclear translocation of the nuclear factor (NF)- $\kappa$ B in D1 cells derived from murine splenic DCs and maintained *in vitro* as growth factor-dependent immature DCs. Inhibition of NF- $\kappa$ B blocked maturation of DCs (as assessed by down-regulation of I-A<sup>d</sup>/I-E<sup>d</sup> MHC II and B7-2/CD86). Additionally, LPS activated ERK and the specific inhibition of MEK1 (the kinase activating ERK) abrogated the ability of LPS to prevent apoptosis, but did not inhibit DCs maturation or NF- $\kappa$ B nuclear translocation. Based on these data, it may be suggested that in LPS-activated DCs, ERK is involved in DCs survival, whereas NF- $\kappa$ B participates in DCs maturation. Similarly, Ardeschna et al. [35] demonstrated that LPS-induced maturation of human monocyte-derived DCs involved activation of p38, ERK, phosphoinositide 3-OH kinase (PI3 kinase)/Akt, and NF- $\kappa$ B pathways. Inhibition of p38 prevented activation of ATF-2 and cAMP response element-binding protein (CREB) and significantly reduced the LPS-induced up-regulation of CD80, CD83, and CD86, but did not have any significant effect on the LPS-induced changes in macropinocytosis or HLA-DR, CD40, and CD1a expression. Inhibiting the NF- $\kappa$ B pathway significantly reduced the LPS-induced up-regulation of HLA-DR as well as CD80, CD83, and CD86. It is therefore quite likely that up-regulation of CD80, CD83, and CD86 by LPS is mediated by at least 2 signal transduction pathways (up-regulation of HLA-DR being NF- $\kappa$ B-dependent and p38-independent). These results corroborate, to some extent, the observations made by Arrighi et al. [26], who also reported blocking the up-regulation of CD80, CD83, and CD86 molecules by SB203580 on DCs stimulated with NiSO<sub>4</sub>. No effects of the inhibitor on up-regulated HLA-DR molecules, observed in this study may suggest p38-independent activation of NF- $\kappa$ B. It may be speculated further that p38-induced expression of costimulatory molecules on DCs exposed to haptens can be mediated by ATF-2 and CREB. In the promoter sequences of CD86 and CD80 genes, at least one binding site can be found for ATF-2 and CREB, and one for CREB, respectively [36,37].



The Janus kinases/signal transducers and activators of transcription (JAK/STAT) signaling pathway in DCs activation by haptens is rather unlikely. Recently, Valk et al. [38] showed that in human monocytes and human monocyte-derived DCs stimulated with MCI/MI, thimerosal, TNCB and formaldehyde there was no significant increase in the phosphorylation of STAT class of transcription factors: STAT1, STAT3, STAT4, STAT5 and STAT6.

## CONCLUSIONS

Accumulated data point to the ability of contact sensitizers to induce maturation/activation of different subpopulations of DCs. An important role in this process is ascribed to interactions of haptens with cellular thiol groups. Up-regulated antigens on DCs, including MHC class II and costimulatory molecules, facilitate activation of T lymphocytes in regional lymph nodes. Down-regulation of molecules, including E-cadherin, is thought to play an important role in releasing DCs from the site of inflammation and triggering their migration to lymph nodes. Although the exact signal transduction pathways of DCs activation by haptens are not known, some papers document elevated levels of phosphotyrosine and activation of mitogen-activated protein kinases (the JAK/STAT signaling pathway probably does not play any important role). It seems that the most important class of MAPK involved in DCs activation is p38 MAPK, although a slight activation of ERK1/2 was also observed. Works conducted so far on p38 MAPK phosphorylation in DCs suggest this endpoint as a useful tool for the screening of potential skin contact sensitizers. Some data indicate that phenotypic changes in DCs induced by various haptens may be different and characteristic of particular hapten. Known ability of haptens to induce DCs maturation/activation and/or release of IL-1 $\beta$  point to some similarities between their mechanisms of activation and activation by common stimulatory factors. Based on some indirect observations, it might be speculated that haptens activate different transcription factors, such as NF- $\kappa$ B, ATF-2 or CREB. Obviously, more work is required to understand better transduction pathways of DCs activation by contact sensitizers. This knowl-

edge would be of great value in designing specific drugs useful in the therapy of contact hypersensitivity.

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