

VOIES DE SIGNALISATION DES HAPTENES CHIMIQUES DANS LA CELLULE DENDRITIQUE HUMAINE

Marc Pallardy, Saadia Kerdine-Römer, Diane Antonios, Philippe Rousseau, Nadège Ade, Zeina El-Ali, INSERM UMR 996, Université Paris-Sud, Faculté de Pharmacie, Rue JB Clément, 92290 Châtenay-Malabry.

Contact : marc.pallardy@u-psud.fr

Sensitisation by chemical resulting in allergic reactions such as contact dermatitis and asthma is an important health issue. Allergic diseases affect up to 20% of the population in the developed countries. In the workplace, irritant and allergic contact dermatitis account for about 40% of occupational illnesses. Many hundreds of chemicals are known to have the potential to cause respiratory or skin sensitisation (albeit with widely varying potencies), and there is a need to understand likely risks to human health and the mechanism of chemical sensitization. The sequence of events has been well characterized for skin sensitizing chemicals and can be summarised briefly as follows. For immunological recognition, and for stimulation of an immune response, a skin sensitising chemical must usually form a stable association with protein. It is believed that in most instances the hapten-protein conjugate is recognised and internalised by dendritic cells (DC) that are found in the dermis and in the epidermis (Langerhans cells). These cells, under the regulation of cutaneous cytokines such as TNF- α and IL-1 β and signals produced by keratinocytes in the microenvironnement, are mobilised and induced to migrate from the skin, via afferent lymphatics, to regional lymph nodes where they locate within the paracortex. Allergen-specific T lymphocytes are then activated and stimulated to divide and differentiate. During *in vivo* immune responses, the role of antigen-presenting cells is played primarily by DC acting as initiators, stimulators and regulators of Ag-specific T lymphocytes. Taking the example of skin sensitization, DC can thus be considered as sentinels of the adaptive immune system, with responsibility for sampling skin surfaces for changes in the antigenic microenvironment. Although the view is that triggering of DC activation requires appropriate danger signals (proinflammatory cytokines, TLR agonists), in addition to experience of new antigens *per se*, a case can nevertheless be made that effective skin sensitisation will be associated with, and dependent upon, the interaction between chemical allergens and resident DC or inflammatory DC differentiated from monocytes upon inflammatory signals.

As mentioned above, it is also now well-accepted that DCs need to receive signals from their environment to migrate and to present antigens to T lymphocytes located in the lymph nodes. These signals are mainly provided through Toll-like receptors that recognize specific structures of microbes or through pro-inflammatory cytokines. In the case of chemical sensitizers, the current hypothesis is that the chemical itself could provide a specific signal to the DC allowing its maturation. Previous works from our group and others have described the activation of MAPK and NF- κ B by chemical sensitizers and their respective roles in DC maturation.

In view of the results recently obtained, we decided to reconsider the question of chemical sensitizers signaling in DC. First, by using a more global approach that will allow us to identify upstream signals activated by chemical sensitizers, second, to integrate the role of electrophilic and oxidative stress in DC maturation induced by these chemicals and third to take advantage of the intriguing effects of metallic haptens on DCs.

Two cell models were used:

- human DC obtained from cord blood CD34+ progenitors. CD34+ cells differentiated in DC using GM-CSF, TNF- α , Flt-3l and SCF (stem cell factor) for 6 days. After 6 days, IL-4 is added for 2 days to obtain more CD1a+ cells.
- human DC derived from monocytes using GM-CSF and IL-4 for 5 days (Mo-DC).

To characterize signalling pathways activated in human DC, the PepChipKinase technology/kinome analysis (Pepscan systems) was used in response to chemical haptens. The PepChipKinase technology allow kinome analysis (the activity of all kinases in whole cell lysates) using in vitro phosphorylation with [γ -33P]ATP arrays consisting of 1176 specific kinases pseudo-substrates.

Results

Phosphokinome assay and identification of kinases activated by chemical sensitizers

Phosphokinome analysis of kinase activated in Mo-DCs allowed us to identify several kinases that were activated by chemical sensitizers (see under an example).

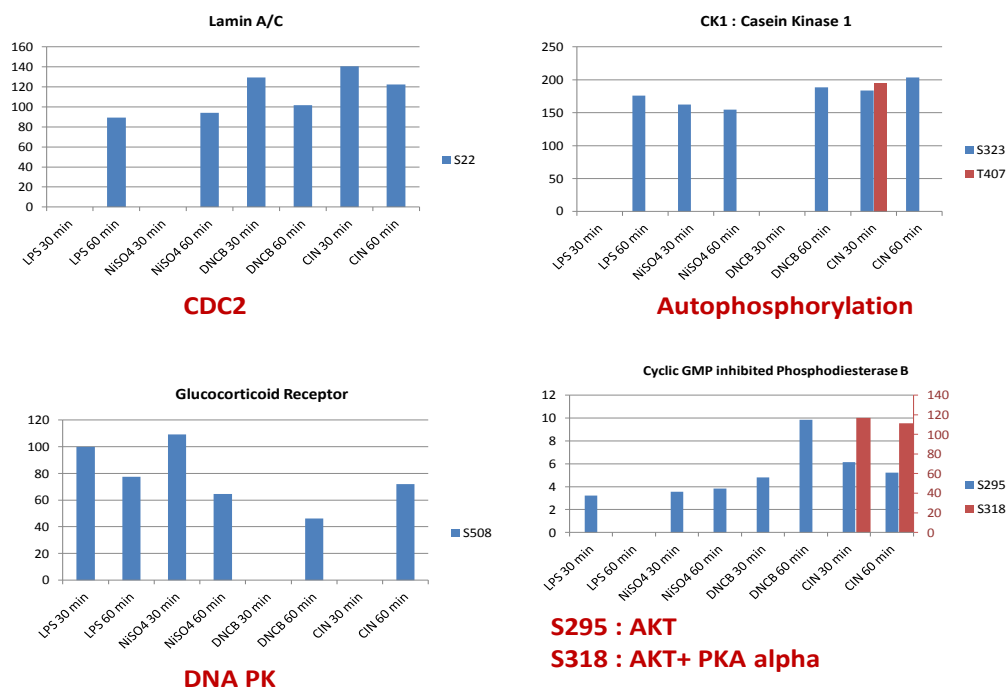


Figure 1: Examples of kinases (in red) activated by NiSO₄, DNCB (dinitrochlorobenzene) or cinnamaldehyde (CIN). In black: name of the substrate used in this setting. Results are expressed as fold-factor

NiSO₄ activates the IRF-1 pathway leading to IL-12 production in DC

In this part of the project, we address the question whether the sensitizer nickel sulfate (NiSO₄) itself or in synergy with other signals can induce the secretion of IL-12p70 in human monocyte-derived DCs (Mo-DCs). We found that IL-12p40 was produced by Mo-DC in response to NiSO₄ stimulation. Addition of IFN- γ concomitantly to NiSO₄ leads to IL-12p70 synthesis. NiSO₄ treatment leads to the activation of MAPK, NF- κ B pathways, and IFN regulatory factor 1 (IRF-1). We investigated the role of these signaling pathways in IL-12 production using known pharmacological inhibitors of MAPK and NF- κ B pathways and RNA interference-mediated silencing of IRF-1. Our results showed that p38 MAPK, NF- κ B, and IRF-1 were involved in IL-12p40 production induced by NiSO₄. Moreover, IRF-1 silencing nearly totally abrogated IL-12p40 and IL-12p70 production provoked by NiSO₄ and IFN- γ . In response to NiSO₄, we observed that STAT-1 was phosphorylated on both serine and tyrosine residues and participated to NiSO₄-induced IRF-1 activation. N-acetylcysteine abolished STAT-1 phosphorylation, suggesting that STAT-1 activation may be dependent on NiSO₄-induced alteration of the redox status of the cell. These results indicate that p38 MAPK, NF- κ B, and IRF-1 are activated by NiSO₄ in Mo-DC and cooperate for IL-12 production.

Metallic haptens induce differential phenotype of human dendritic cells through activation of mitogen-activated protein kinase and NF- κ B pathways

Metallic haptens are able to induce DC maturation in vitro but the mechanism of this maturation is not well understood. We and others have already shown that NiSO₄ activates p38 mitogen-activated protein kinases (p38MAPK), c-jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and the transcription factor NF- κ B during the early events of DCs maturation. However, the effect of other metallic haptens on DC maturation is still poorly understood. In the present study, using dendritic cells derived from CD34⁺ cord blood cells, we showed that both NiSO₄ and CoCl₂ induced the expression of CD86, CD83, HLA-DR and CD40 and the production of IL-6 in human DCs while K₂Cr₂O₇ induced only a slight upregulation of CD86. Interestingly, only NiSO₄ was able to induce the production of IL-12p40. NiSO₄ and CoCl₂ but not K₂Cr₂O₇ were able to activate the MAPK pathway and the transcription factor NF- κ B. The role of MAPKs in metals-induced DC maturation was then evaluated using well-described pharmacological inhibitors. Our results suggest that p38MAPK activation regulates the expression of CD86 and CD83 induced by NiSO₄ while it only affects the expression of CD83 induced by CoCl₂. IL-6 production induced by NiSO₄ and CoCl₂ strongly depended on all MAPKs. IL-12p40 synthesis after NiSO₄ treatment was regulated by both p38MAPK and JNK pathways whereas ERK may play an

inhibitory role. Our results show that both NiSO₄ and CoCl₂ activate similar signaling pathways that are playing different roles in DC maturation depending on the hapten used.

Role of the electrophilic and Redox stress in DC activation by chemical sensitizers

Electrophilicity is one of the most common features of skin contact sensitizers and is necessary for protein haptentation. The Keap1 (Kelch-like ECH-associated protein 1)/Nrf2 – signaling pathway is dedicated to the detection of electrophilic stress in cells leading to the upregulation of genes involved in protection or neutralization of chemical reactive species. Signals provided by chemical stress could play an important role in dendritic cell activation and the aim of this work was to test whether contact sensitizers were specific activators of the Keap1/Nrf2 pathway. CD34-derived dendritic cells (CD34-DC) were treated by a panel of sensitizers (NiSO₄, 1-chloro 2,4-dinitrobenzene, cinnamaldehyde) and expression of *hmox1* and *nqo1* as Nrf-2 target genes was measured using real-time PCR and cellular accumulation of Nrf2 was assessed by Western blot. Our results showed an increased expression at early time points of *hmox1* and *nqo1* mRNAs in response to sensitizers but not to irritants. Accumulation of the Nrf2 protein was also observed only with chemical sensitizers. Altogether, these data suggested that the Keap1/Nrf2-signaling pathway was activated by electrophilic molecules including sensitizers in dendritic cells.

In conclusion, our study identified the Nrf2 pathway as a new signalling pathway activated by chemical sensitizers. It is our opinion that activation of this pathway may provide the possibility for DC to handle the electrophilic stress induced by chemical sensitizers allowing DC maturation by activation of MAPK and NF-κB. Future studies will be conducted to address this hypothesis. In addition, genes regulated by Nrf2 such as *hmox1* and measurement of Nrf2 protein accumulation are good candidates for new biomarkers for chemical sensitizer detection in cell-based models.

Publications

- Ade N, Leon F, Pallardy M, Peiffer JL, Kerdine-Romer S, Tissier MH, Bonnet PA, Fabre I, Ourlin JC. HMOX1 and NQO1 genes are up-regulated in response to contact sensitizers in dendritic cells and THP-1 cell line: role of the Keap1/Nrf2 pathway. *Toxicol Sci*, 107 (2):451-60 (2009).
- Antonios D, Ade N, Kerdine-Römer S, Assaf-Vandecasteele H, Larangé A, Azouri H, Pallardy M. Metallic haptens induce differential phenotype of human dendritic cells through activation of mitogen-activated protein kinase and NF-KB pathways. *Toxicol In Vitro*, 23(2):227-234 (2009)
- Antonios D, Rousseau P, Larangé A, Kerdine-Römer S and Pallardy M. Mechanisms of IL-12 synthesis by human dendritic cells treated with the chemical sensitizer NiSO₄. *J Immunol*. 185(1):89-98 (2010)

Conférences invitées

- « NF-KB plays a major role in the maturation of dendritic cells by chemical sensitizers ». Symposia « Transcriptional changes in immunotoxicology : Transcription factors signal transduction and epigenetics ». 48th annual meeting of the Society of Toxicology. Baltimore. USA. March 2009.
- “Use of dendritic cells for the identification and characterization of chemical allergens”. Symposia “Chemical sensitization: from immunobiology to quantitative risk assessment” 46th EUROTOX meeting, Dresden, Germany, September 2009.

Communications orales

- « Heme-oxygenase 1 and NADPH Quinone oxidoreductase 1: new targets genes to predict the sensitising potential of chemicals ? ». 47th annual meeting of the Society of Toxicology. Seattle. USA. Platform session “Mechanisms of hypersensitivity”. March 2008.
- The chemical sensitizer nickel sulfate induces the production of interleukin-12: cooperation among p38 MAPK, NF-KB and IRF-1 pathways. 48th annual meeting of the Society of Toxicology. Baltimore. USA. Platform session “Mechanisms of hypersensitivity”. March 2009.