Summary

Dendritic cells (DC), known as professional antigen presenting cells (APC), play an important role in the modulation of the immune responses. They take up, process and present antigens to naïve T lymphocytes, which start proliferating and differentiate into effector cells. In peripheral tissues, resident DC exist in an immature state but they are reactive to signals characteristic of inflammation or infection. Those signals include the endotoxin lipopolysaccharide (LPS), double stranded viral RNA, unmethylated motifs of cytosine and guanine (CpG) from bacterial DNA and inflammatory cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1 and IL-2. After exposure to those signals, DC undergo a process of maturation, characterized by production of cytokines, including chemokines and IL-12, increase in the expression of costimulatory molecules involved in antigen presentation to lymphocytes (CD40, CD80, CD86, DC-SIGN), and increase in the expression of proteins of the major histocompatibility complex (MHC). This process of maturation occurs simultaneously with the migration of dendritic cells from peripheral tissues to lymph nodes, where they activate naïve lymphocytes, thus ensuring the initiation and the fate of the immunological response.

Although some phenotypical and functional modifications occurring during DC migration and maturation have already been identified, the intracellular signalling pathways activated in those biological events are not completely understood. Nitric oxide (NO), produced by the inducible isoform of nitric oxide synthase (iNOS), regulates dendritic cell functions, namely antigen uptake and presentation to T lymphocytes. The expression of iNOS protein is dependent on transcription nuclear factor *kappa* B (NF-κB) and several studies demonstrate the involvement of this transcription factor in DC differentiation, maturation and survival.

The aim of this work was to study the signalling pathways activated in DC after exposure to LPS, cytokines and contact sensitizers, namely those involved in iNOS expression. As an experimental model of a dendritic cell we used a fetal skin-derived dendritic cell line, which has morphological, phenotypical and functional characteristics of skin dendritic cells, the Langerhans cells (LC).

Therefore, in this work we studied the effect of LPS and granulocyte macrophage-colony stimulating factor (GM-CSF) on iNOS expression and NO production by FSDC cells. We also investigated the intracellular signalling pathways, activated by LPS and GM-CSF, which are involved in iNOS expression. Our results showed that LPS and GM-CSF increased iNOS protein expression and NO production by FSDC cells, being LPS the most potent stimuli. iNOS expression elicited by LPS and GM-CSF involved Janus kinase 2 (JAK2) and NF-κB activation.

The effect of two contact sensitizers, 2,4-dinitrofluorbenzeno (DNFB) and nickel, on iNOS expression and NO production by FSDC cells was also investigated. Moreover, we studied whether those contact sensitizers activate the transcription factor NF-κB. Our results showed that the contact sensitizer nickel, but not DNFB, increased iNOS protein expression and NO production by FSDC cells. Both sensitizers induced NF-κB binding to DNA but there was a differential activation of the NF-κB subunits in response to DNFB and nickel.

A better knowledge of the cellular and molecular events that occur during dendritc cell differentiation and maturation will allow: i) to develop and improve immunotherapeutic strategies that manipulate the immune response against cancer and infectious diseases; ii) to develop *in vitro* alternative approaches for identification of the allergenic potential of environment substances, which is currently evaluated using experimental animals.