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In vitro prediction of contact sensitization: simultaneous evaluation of dendritic cells and of T lymphocytes in a coculture system

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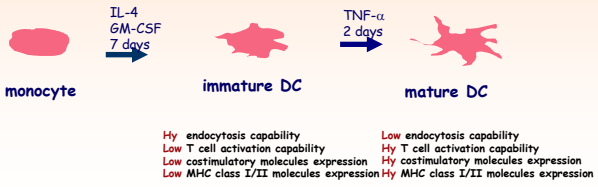
Introduction

Nickel is the most important sensitizing agent involved in allergic contact dermatitis (ACD). Despite of the clinical relevance of Nickel ACD among the population, the molecular mechanism which triggers the biological response and the inflammatory reaction following Ni exposure is yet to be understood. Furthermore there is a well established need for the investigation and development of *in vitro* methods able to predict the contact sensitization potential of topical products and chemical substances.
Dendritic Cells (DCs) are specialized antigen presenting cells which provide the fundamental framework for T lymphocyte activation. Skin sensitization to metals depends on the activation of T lymphocytes, which involves both $CD4^+$ and $CD8^+$ T cells. Moreover, a modest but consistent intrinsic activatory potential of $NiSO_4$ has long been described, both in sensitized and in non sensitized individuals.
Here we used a well established in vitro system to study the effect of the prototypic skin sensitizer $NiSO_4$ on monocyte derived DCs **(Fig.1)** .

Fig. 1: Human monocyte-derived Dendritic Cells



Materials and methods

Preparation od DCs.
Peripheral blood mononuclear cells were isolated from the blood of healthy donors (kindly provided by the blood transfusion department of San Raffele Hospital) with a density Fycoll-Paque gradient as previoulsy described. Mononuclear cells were resuspended in RPMI, 10% fetal calf serum, and allowed to adhere to 6-well plates (Costar, Cambridge, MA). After 2 h at 37 °C non-adherent cells were removed. Monocytes were then cultured for 7 days in RPMI containing 10% fetal calf serum, 100 µg/ml penicillin, 100 µg/ml streptomycin, human GM-CSF (50 ng/ml), and IL-4 (1000 units/ml) to derive immature DC. Mature DC cells were obtained from immature DC by a 48-h incubation in RPMI containing 10% fetal calf serum, 100 µg/ml penicillin, 100 µg/ml streptomycin in the presence of human TNF (0.2-200 ng/ml). In order to evaluate the effect of Ni on the maturation of DCs, $NiSO_4 \cdot 6H_2O$ was added at different concentration for the same incubation time.

FACS staining
The maturation of the single DC preparations was routinely assessed by flow cytometry, measuring the exposure on the plasma membrane of specific antigens known to be expressed by immature or mature DC, namely: CD1a, a marker of human myeloid DC; MHC class I and class II molecules; CD80 (B7.1), CD86 (B7.2), and CD83, involved in T cell co-stimulation. Expression of these antigens was analyzed after staining with appropriate FITC-labeled Abs , using a fluorescence-activated cell sorter (FACStar Plus, Becton Dickinson, Sunnyvale, CA). **Fig. 2** shows results from a typical analysis.

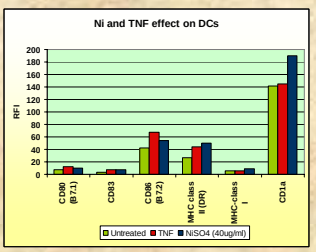
Lymulus Amebocyte Lysate (LAL) Assay.
In order to exclude that the effect of $NiSO_4$ on immature DC could be ascribed to bacterial contaminants in the sample, a LAL Assay was performed according to the Manufacturer's instructions (Pyrotell, Associated of Cape Cod Incorporated, Falmouth, MA).

MTT assay.
In order to evaluate the toxic effect of NiSO4 on human DCs, a standard MTT assay was performed. MTT-medium is prepared by adding 15 mg of MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) to 30 ml of culture medium.
After exposure of the cells to the test material, the cells are washed with PBS. 200 µl of MTT-medium is added to each culture well and the cells are incubated for 4 hours at 37°C.The MTT-medium is than removed and 200 µl / well of MTT Solubilization Solution (10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol) is added to the cells. After 30' of incubation with shaking, the absorbance is measured at 540 nm on a microplate reader, with background reading at 690 nm.

Mixed Lymphocyte Reactions (MLR).
In order to evaluate the modulation in antigen presenting cell capability due to the maturation f DC induced by $NiSO_4$ exposure (or, TNF alpha, as control), classical Mixed Lymphocyte Reactions (MLR) were performed. In brief, responder T lymphocytes from an (allogeneic) non related donor were co-cultured with DCs at different DC to T cell ratios. The read out of the assay was T cell proliferation, as assessed by ^{3}[H]-TdR incorporation in a standard 18-hrs pulse assay.

Results

Fig. 3
Comparison of the pro-differentiative effect of $NiSO_4$ versus TNF alpha.
The relative fluorescence intensity (*RFI*) was measured on DC obtained as above described, in the presence of 40 µg/ml of $NiSO_4$.



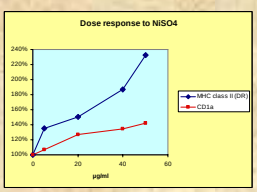
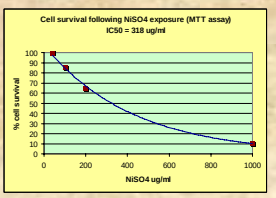


Fig. 4
Dose response effect of $NiSO_4$ on the expression of MHC class II and of CD1a membrane differentiation markers on human DCs. Results are expressed as percent relative fluorescence intensity (*RFI*) versus immature DCs.

Fig.5
Cytotoxicity assay.
Cell survival after 48-hrs exposure to different concentrations of $NiSO_4$, as measured with standard MTT assay.

Lymulus Amebocyte Lysate (LAL) Assay.
Lymulus Amoebocyte Gelification (LAL) Assay yielded a negative result when $NiSO_4$ was assayed at 100 mg/ml concentration (data not shown).



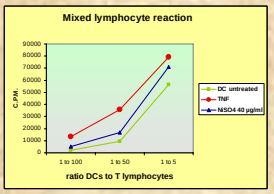
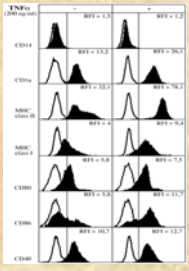


Fig. 6
Mixed Lymphocyte reaction.
Human DCs were co-cultured with peripheral blood T lymphocytes from a non related donor, at different DCs to T cell ratios, as indicated in the abscissa. Undifferentiated DCs were used as target cells, which were either left untreated, TNF alpha treated or $NiSO_4$ treated. On the y axis proliferation is indicated as ^{3}[H]TdR incorporation , as measured by a beta scintillation counter in count per minute (CPM) .

Conclusions

We show here that a prototypic skin sensitizer, $NiSO_4$ can modulate the expression of molecules involved in antigen presentation on human, monocyte derived immature DCs. The effect we observe is extended to several differentiation markers, including CD80, CD83, CD86, CD1a, MHC class I and II and is quantitatively similar to that induced by TNF alpha.
We also found that $NiSO_4$ treated DCs act as better antigen presenting cells in a mixed lymphocyte reaction assay.
In parallel experiments, we did not find any effect on DCs differentiation by other skin sensitizers (DNFB, parabens and other common preservatives)(data not shown).
The results of this study contribute to the comprehension of the complex series of cellular and molecular events which can induce contact hypersensitivity.

Fig.2
Phenotypic characterization of human DC exposed to TNF alpha. Human monocytes were treated with GM-CSF (50 ng/ml) and IL-4 (1000 units/ml) for 7 days to obtain immature DC. These cells were then incubated with or without TNF (200 ng/ml) for a further 48 h (*right* and *left columns*, respectively). Cell preparations were stained with FITC-conjugated Abs for surface antigens, as specified on the *left-hand side*, and analyzed by flow cytometry . Their relative fluorescence intensity (*RFI*) was calculated *versus* negative controls (*open histograms*). The results shown are from one representative experiment.



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