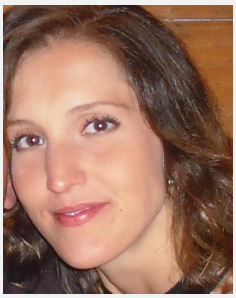
ImmunoTools *special* Award 2015



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Heavy metal toxicity: analysis of cellular and molecular mechanisms underlying the alterations of the innate and adaptive immune responses

Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Heavy metals toxicity can result in damaged or reduced mental and central nervous function, neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are not uncommon and repeated long-term contact with some metals or their compounds may even cause cancer.

The symptoms of toxicity resulting from chronic exposure often develop slowly over months or even years because low-level exposure may cause immune dysfunction by increasing oxidative stress, affecting transmembrane calcium signalling and splenic cellularity (1,3). Chelation therapy is the process by which a molecule encircles and attaches to the metal removing it from tissue. Chelating agents specific to the heavy metal are given orally, intramuscularly, or intravenously. Once the bound metal leaves the tissue, it enters the bloodstream, is filtered from the blood in the kidneys, and then is eliminated in the urine.

However, the chemical drugs currently in use are not so efficient and are uncomfortable because of their side effects (4). $Co2^{+}$ plays a relevant role in inducing hypersensitivity reactions and we have demonstrated that CoCl2 causes slow but progressive apoptosis of human T cells while activating monocytes that produce NF-kB dependent proinflammatory cytokines. However, CoCl2-treated monocytes appeared to lose their antigen-processing/presenting properties displaying a reduced efficiency to capture the antigen as well as to stimulate the alloreactive T cell response (5). PCs are produced in plants, algae and some fungi (6) under heavy metal stress. PCs can form stable complexes with some metals/oids that are subsequently transported into the vacuoles where they are not harmful to the cell. PCs are a mixture of oligomers of different length, called respectively PC2, PC3, PC4

and PC5, whose relative concentrations vary in different tissues and in different species (7). PC accumulation is also induced by $Co2^{+}$ in Silene vulgaris (8) PCs are synthesized from GSH, by phytochelatin synthase (PCS) and might chelate $Co2^{+}$ions in mammalian cells more efficiently than GSH. This would lead to an alternative therapeutic approach since the current therapies rely on metal-detoxifying treatments which are themselves toxic.

Based on these speculations in this proposal we intend to pursue the following major aims:

1. To characterize and correlate the phenotype of the surviving T cells to the immune response involved in the $Co2^{+}$induced inflammation. The following analysis of the TCR sequences of the clones obtained could elucidate whether $Co2^{+}$can act as a superantigen in dependence of the allelic polymorphism (Glu69) of the HLA-DP beta chain associated with Hard Lung Metal Disease (3).
2. To verify whether PCs are functional in $Co2^{+}$chelation in plants and if, in mammalian cells, they can act as antioxidant and detoxifying agents while maintaining cellular homeostasis. To this aim the role of PCs in $Co2^{+}$detoxification will be analysed in the plant model species Arabidopsis thaliana and the PCS gene from Arabidopsis will be expressed in mammalian cells (U937, Jurkat and Monocytes Derived Macrophages treated with different reagents (TPA, ATRA, 1,25dihydroxyvitamin D3, IFN-gamma, M-CSF, GM-CSF, IL-4) to allow PC production.
3. Extending this type of analysis to the study of other toxic heavy metals (e.g. Arsenic, Nickel, Mercury).

In this project, the expression of the following markers and proteins regulated during activation/differentiation will be tested: CD11b, CD14, CD11c, CD14, CD16, CD1a, CD80, CD86, CD32, CD45RO, CD45RA, CD64, CD54, CD102, CD106, HLA-ABC, HLA-DR, HLA DP, CD56, CD3, CD4, CD8 (in multicolor combinations), CD19, CD65, CD29, CD62L, IFN-gamma, IL-6, IL-8, TNFa.

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