

<p>Activity Name:</p> <p><i>In-vitro</i> immunotoxicity studies</p>	<p>Category: D. In-vitro toxicity studies</p> <p>Institute: Slovak Medical University</p> <p>Location: Limbova 12, 833 03 Bratislava, Slovakia</p> <p>Contact Details of Expert:</p> <p>Name: Jana Tulinska, Aurelia Liskova, Miroslava Kuricova, Mira Horvathova</p> <p>Phone: +421 2 59370 244, 830, 242, 540</p> <p>E-mail: jana.tulinska@szu.sk, aurelia.liskova@szu.sk, miroslava.kuricova@szu.sk, mira.horvathova@szu.sk</p>
<p>Short technology description/Overview (<i>approx 300 words</i>):</p> <p>Study of possible interactions between nanoparticles (NP) and the components of the immune system is important part of safety assessment of promising nanomaterials. Direct immunotoxic effect of NP can be the result of cytotoxicity, effect on growth and maturation of immune cells, induction of apoptosis, interaction with signaling processes and others. Moreover, induction of immune responses including profound adjuvant effect on development and intensity of allergic responses may occur as demonstrated by several studies (Riedl et al. 2005). On the other hand, immunotoxic effect can lead to increased susceptibility to infection and cancer as well.</p> <p><i>In vitro</i> studies using fractionated blood products can be utilized in evaluating the effect of NP on circulating blood. Cytokine/chemokine release from leukocytes or complement activation is relevant endpoint to evaluate for NP. The effects of NP on immunological functions, including antigen presentation by macrophages and dendritic cells and the subsequent effects on immune responses <i>in vitro</i> can be evaluated (Oberdörster 2005).</p> <p>Installation for <i>in vitro/and in vivo</i> immunotoxicity studies is available at the Department of Immunology and Immunotoxicology, Slovak Medical University. Laboratory of Immunotoxicology fulfills the requirements requested by Good Laboratory Practices (GLP) regulations for facilities engaged in toxicology testing. Laboratory has certificate of Good Laboratory Practice and Laboratory of Immunology has Accreditation certificate according to the STN EN ISO/IEC 17025:2005.</p> <p>In our laboratories, the following test methods are employed in assessment of immunotoxicity:</p> <p><i>Immunophenotypic analysis of leukocytes</i></p> <p>Method for dividing leukocytes into clonal subgroups on the basis of differences in cell surfaces antigens. The antigenic differences are detected with monoclonal antibodies and flow cytometry. Human blood cells, cells derived from exposed animals or <i>in vitro</i> cultures can be examined.</p> <p><i>Detection of cytokines/chemokines</i></p> <p>Cytokines or chemokines of interest are analyzed by ELISA method using UV/VIS spectrophotometry. Serum/plasma of animals exposed to nanoparticles can be evaluated. For <i>in vitro</i> studies, presence of</p>	

nanoparticles in samples is limitation factor and possible source of interference with method.

Immune function assays:

Phagocytic activity and respiratory burst of leukocytes

The assay evaluates phagocytic activity and respiratory burst of polymorphonuclear neutrophils/ monocytes exposed to the test compound administered *in vivo* in experimental animals or *in vitro in cell* culture systems using fluorescein-labelled Staphylococcus aureus and hydroxyethidine and measured by flow cytometry.

Lymphocyte transformation test

It is an *in vitro* test of lymphocyte function. The test examine increased DNA synthesis (³H-thymidine incorporation) followed by cell division and differentiation of lymphocytes in response to antigens or mitogens using liquid scintillation method.

Human blood cells *in vitro* pulsed with nanoparticles or spleen/blood lymphocytes derived from exposed animals can be examined.

Nanoparticles:

In our lab, we used immune assays for evaluation of the potential immunotoxic effect of nanoparticles: titanium dioxide (TiO₂), poly (D, L-lactide-co-glycolide) (PLGA), silica (SiO₂), Endorem, uncoated magnetite (Fe₃O₄) and sodium oleate coated magnetite. Human peripheral blood leukocytes were exposed with nanoparticles *in vitro*. Rat animal model was dosed with nanoparticles *in vivo* intravenously.

Test systems: human peripheral blood leukocytes, laboratory animals – rats/mice/guinea pigs (OECD 406, OECD 407, OECD 429)

Main Features (Equipment Capabilities):

- Flow cytometer - Epics XL Beckman Coulter
- UV/VIS spectrophotometer
- Liquid scintillation counter - Microbeta 2 (Perkin Elmer)
- Hematology analyzer - Sysmex K 4500
- Biohazard laminar box
- Centrifuge - Hettich
- Incubator with CO₂ atmosphere - Jouan, Heracell
- Light microscope - Leitz

Typical Samples & Images:



Any further Information: