

CHARACTERIZATION OF IMMUNE REACTIONS TO METALS

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Metals and their ions are released from dental materials, orthopedic implants, they originate from drugs and vaccines or are inhaled and ingested. Intoxications through environmental or workplace exposure to high concentrations of metals are rare events. In general, metal associated health problems are more related to long-term internal or external exposure to lower, subtoxic concentrations. In recent years mercury has been a major focus of interest and of controversies. By some, mercury is regarded as a major health problem. Another heavy metal, nickel, is unequivocally the number one contact allergen with a sensitization rate of up to 25% among the female population. Dental amalgam is the major source of mercury, except in fish consuming populations where the lipid-soluble, highly neurotoxic methylated derivative of mercury is the prevailing source. Besides prolonged exposure there are obviously genetic susceptibility factors important in the development of individual intolerance reactions. There is, for instance, increasing knowledge about individual differences in the metabolism and elimination of xenobiotics, toxic metabolites and metals as well. Genetic polymorphisms and differences in gene expression result in highly variable activities of detoxification enzymes, some of which – like the glutathione-S-transferases, are also relevant for the elimination of metals. These and other individual factors are determinants of individually different patterns of reaction to otherwise identical metal concentrations. The most relevant determinant of xenobiotic tolerance, even in very low concentrations, is the immune system. It serves as a sensor and filter for all kinds of external materials entering the organism via the respiratory tract, the skin or the digestive system. In animal models many basic principles of immune reactions to metals have been established. For mercury in particular a comprehensive concept of the immunological reactions leading to the autoimmune glomerulonephritis in genetically susceptible rodents, has been established and the potential self antigen fibrillarlin has been identified. But these and other results of animal studies on metal intolerance may not be relevant to humans. Thus mercury while provoking non-organ specific autoantibodies obviously does not induce autoimmune diseases in humans and a mercury-specific autoantigen has not been identified. In-vitro two completely different types of immune reactions to mercury can be distinguished. Higher, supranormal concentrations of metals induce cellular stress reactions, characterized by the generation of free radicals and a profound drop in antioxidative defense mechanisms. Consecutive destabilization of the cellular redox balance results in an inflammatory type immune reaction via activation of NF- κ B related, redox-sensitive transfer factors. Among

the NF- κ B dependant reactions are the increased expression of heat-shock proteins like Hsp 70, and of the TH1-type pro-inflammatory cytokine interferon gamma. Metals/metal ions like titanium that are water insoluble may induce that type of particle-dependant inflammatory response. Finally, in genetically susceptible patients low concentrations of metal ions can induce metal hapten-specific T cells and an interleukin 2 triggered proliferative cellular immune response of delayed type, a type IV allergy. The probable carrier molecules forming a complete antigen with the metal hapten have not been identified so far.

The specific type of cellular immune reaction to metals is not suitable as a general tolerance test since it will only be established after exposure to the particular metal. Although the knowledge of the frequency of delayed type immune reactions to certain metals can be of help. The unspecific inflammatory immune reaction to metals on the other hand could serve as a valuable in-vitro technique in establishing the biological tolerance threshold for metals in humans. For this purpose our group has developed an in-vitro test system for the characterization of individual xenobiotic tolerance thresholds. This so-called **Immune Tolerance Test (ITT)[®]** has enabled for the first time the diagnosis of multiple chemical sensitivity (MCS) by in vitro exposure of patient lymphocytes to low concentration test chemicals. The established test procedures for type IV allergies are the patch test and as in vitro test the lymphocyte transformation test (LTT), both with specific advantages and also some drawbacks. The skin tests are prone by the prevalence of unspecific irritative reactions, the chance of test-induced sensitization and lack of specificity for a number of chemicals including metals. The LTT has been greatly improved in recent years. A new, highly specific and sensitive version of the test has been developed by our group, called **LTT-CITA[®]** (Cytokine Improved Transformation Assay) and has been introduced for the assay of xenobiotic allergies in general and metal allergies in particular. It has proved its value also for protein antigens from food, bacteria, viruses or moulds.

The individuality of the cellular immune response to chemical antigens can be further dissected by characterizing the effector response in vitro at the level of cytokine gene expression, allowing a high throughput and fast way of testing (**Effector Gene Test**). Cellular immune reactions to metals may either belong to the Th1 type with interferon-gamma being the prominent cytokine expressed after incubation of the lymphocytes with the antigen or to type Th2 with interleukin 10 as the major reactant. If interleukin 2 is also expressed a broad proliferative response to the particular antigen can be expected, resembling the specific delayed-type immune reactivity.