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Allergy and Autoimmunity Caused by Metals: A Unifying Concept

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Introduction

Allergy and autoimmunity are caused by an abnormal immune response and have the same clinical outcomes, including local and systemic inflammation resembling autoimmune/inflammatory syndrome induced by adjuvants (ASIA) (Shoenfeld and Agmon-Levin, 2011; Perricone *et al.*, 2013).

This chapter will give an overview of the literature on metal-induced pathologies, such as delayed-type hypersensitivity and autoimmunity. Because of the vast amount of information available on this subject, the focus of this review will be mainly on specific T cell reactivity to mercury, aluminum, nickel, and gold, all of which are known to induce immunotoxic effects in human subjects. Mercury, as a constituent of thimerosal, and aluminum are both used in vaccines.

The immunological effects of metals include immunomodulation, allergy, and autoimmunity. Metals may act as immunosuppressants or as immune adjuvants. One example of immunomodulation is the ability of metals to modify cytokine production *in vitro* and *in vivo*.

In the body, metal ions may firmly bind to cells and proteins. This binding results in the modification of autologous epitopes (i.e. haptenization). In susceptible individuals, T cells falsely recognize the modified proteins as foreign and start an autoimmune attack (Griem and Gleichmann, 1995; Schiraldi and Monestier, 2009; Wang and Dai, 2013). In experimental animals, the recognition of metal haptens is dependent on the genetic makeup: some rodent strains are resistant, while others are susceptible to the induction of autoimmunity by metals (Griem and Gleichmann, 1995; Bigazzi, 1999; Fournié *et al.*, 2001; Schiraldi and Monestier, 2009). Clusters of autoimmunity have been reported in areas of increased exposure to heavy metals (Ingalls, 1986). It has been found that mercury, nickel, cadmium, lead, aluminum, and arsenic can exert immunotoxic effects through epigenetic mechanisms, such as DNA methylation and histone modification (Greer and McCombe, 2012).

In humans, the expression of autoimmune diseases can differ between genetically identical twins. This suggests that, in addition to genetics, environmental factors are involved in the disease process. The genes controlling susceptibility to metals are the subject of intensive studies (Wang *et al.*, 2012; Woods *et al.*, 2013), but no clear conclusion has yet been reached. Genes that might predispose for toxic effects of metals are, for example, those involved in detoxification and synthesis of glutathione. In the case of metal allergy, only a few genetic studies have been performed, such as those on workers occupationally sensitized to beryllium (Wang and Dai, 2013).

Delayed-type hypersensitivity

The type of allergy induced by metals in humans is cellular-type hypersensitivity, also called type IV delayed-type hypersensitivity. "Delayed" refers to

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the fact the first symptoms appear 24–48 hours after initial exposure to the allergen, which makes causal connection difficult. Metals such as mercury are low-molecular haptens and only rarely produce antibodies (Wylie *et al.*, 1992). Hence, immunological responses induced by metals are mostly T cell-mediated.

The gold standard for diagnosis of delayed-type hypersensitivity is patch testing. In patch test, the suspected metal allergens are applied under occlusion on the skin of the back. A dermatologist evaluates the reaction after 2–3 days. Another diagnostic approach, one that is becoming more widespread, is the lymphocyte transformation test (LTT), which allows an objective evaluation of memory lymphocytes present in the blood of patients. In this test, blood lymphocytes are cultivated with metals or other allergens for 5 days *in vitro*, after which the number of proliferating lymphocytes is determined by radioisotope incorporation.

A standardized and validated form of LTT is LTT-MELISA (Memory Lymphocyte Stimulation Assay) (Stejskal *et al.*, 1994, 2006; Prochazkova *et al.*, 2004; Valentine-Thon *et al.*, 2007). In addition to objective radioisotope evaluation, morphological confirmation of the presence of activated lymphocytes (lymphoblasts) is also performed (Stejskal *et al.*, 2006).

The allergic and autoimmune effects of metals

Exposure to metals can be external (e.g. through pollution, occupation, cosmetics, and handling of metallic items) or internal (e.g. through foods, dental restorations, orthopaedic implants, and vaccines). Cigarette smoke contains many metals, such as mercury, cadmium, lead, arsenic, and nickel, and increasing evidence is linking it to autoimmune disorders (Arnson *et al.*, 2010).

Mercury

It has been known for decades that exposure to mercury through skin-lightening ointments will, in some individuals, lead to the development of serious side effects, such as kidney disease (Turk and Baker, 1968; Barr *et al.*, 1972; Kibukamusoke *et al.*, 1974), as well as neurological complications such as peripheral polyneuropathy (Kern *et al.*, 1991; Adawe and Oberg, 2013). In a more recent paper, skin-lightening creams induced neuropsychological problems and glomerulonephritis in a patient with juvenile diabetes (Pelcova *et al.*, 2002). After mercury chelation, the symptoms disappeared, confirming a causal relationship. Mercury-containing ointments are still being used in some countries (Weldon *et al.*, 2000).

The main source of inorganic mercury in the general population is mercury released from dental amalgam fillings (Clarkson *et al.*, 1988). Dental amalgam consists of 50% mercury, ~22–32% silver, ~14% tin, ~8% copper, and other trace metals (Ferracane, 2001). Since mercury functions as both adjuvant and allergen, it has no safe dose level (IPCS, 1991). The most common source of methyl mercury is ingested polluted fish. Methyl mercury can also be formed through the conversion of metallic mercury by oral and gastrointestinal bacteria, and vice versa (Liang and Brooks, 1995).

Thimerosal and phenyl mercury are organic mercury compounds used as antiseptics and preservatives in eye drops and vaccines (Rietschel and Fowler, 2001). Like methyl mercury, these organic mercury compounds are decomposed to inorganic mercury in the body (WHO, 1990; Havarinasab and Hultman, 2005).

Inorganic mercury, thimerosal, and nickel are the most common allergens in children, a fact that is not widely recognized. Of 1094 children with skin disease, 10% reacted to thimerosal (ethylmercury thiosalicylate) and 6% to mercury (Seidenari *et al.*, 2005) in patch test. A review of PubMed articles investigating allergens in at least 100 children from the years 1966–2010 showed that among the top five allergens across 49 studies, three were metals: nickel, gold, and thimerosal (Bonitsis *et al.*, 2011).

Sensitization to thimerosal can be demonstrated *in vitro* by LTT-MELISA, as shown previously (Stejskal *et al.*, 1994, 1999; Stejskal, 2014). In a large study of over 3000 patients, tested by LTT-MELISA in three different laboratories, the prevalence of thimerosal-specific lymphocyte responses was around 7% (Stejskal *et al.*, 1999). As shown in Table 5.1, LTT-MELISA can identify thimerosal-specific responses in patients who have experienced side effects after exposure to thimerosal-containing products.

According to one paper (Westphal *et al.*, 2000), thimerosal sensitization depends on homozygous gene deletion of the glutathione S-transferases, indicating the role of genetics in detoxification capacity.

It is important to note that memory lymphocytes induced by various mercury compounds do not crossreact, as shown by Italian dermatologists (Tosti *et al.*, 1989; Santucci *et al.*, 1998) and by

Table 5.1 Lymphocyte responses in LTT-MELISA to thimerosal and other metals in patients with side effects following exposure to thimerosal-containing products

Patient number	Sex	Age	Health status	Thimerosal exposure	Symptoms after exposure	Positive thimerosal responses (SI)	Other positive responses
1	F	45	CFS	Hepatitis-B vaccine, gamma globulin	Flu-like symptoms after hepatitis B vaccine	**20**	Cadmium, palladium, phenyl mercury, tin
2	F	52	Skin/eye irritation, fatigue	Anti-D globulin × 3, eye drops, TB vaccine, patch test	Worsening of symptoms after thimerosal patch testing	**19**	Ethyl mercury, inorganic mercury, methyl mercury
3	F	58	CFS	Vaccines	Flu-like symptoms post-vaccination	5.9	Inorganic mercury, phenyl mercury
4	F	53	CFS, oral lichen planus	Gamma globulin × 8, cosmetics	Eyelid eczema and edema from cosmetics	**41**	None
5	F	48	CFS	Vaccines	Not known	7.3	None
6	F	18	Heart problems	Vaccines	Not known	**16.3**	Cadmium, copper, inorganic mercury, lead, methyl mercury, phenyl mercury
7	F	57	CFS	Gamma globulin, TB vaccine	Not known	**65**	None
8	F	45	CFS	Vaccines	Not known	**12.4**	Ethyl mercury, gold, inorganic mercury, lead, methyl mercury, nickel, phenyl mercury, tin
9	M	47	CFS	Gamma globulin, eye drops	Not known	4.4	Cadmium, ethyl mercury, gold, inorganic mercury, lead, methyl mercury, nickel, palladium, phenyl mercury, tin
10	F	53	CFS	Gamma globulin, eye drops	Not known	4.4	Cadmium, ethyl mercury, methyl mercury, nickel

Lymphocytes were isolated from human blood and cultivated for 5 days with a wide range of metal salts, including thimerosal, inorganic mercury, methyl mercury, phenyl mercury, gold, palladium, tin, lead, nickel, and cadmium (Stejskal *et al.*, 1999). Metal-specific responses were measured by 3H thymidine uptake. Lymphocyte responses are shown as stimulation index (SI) = counts per minute (cpm) in metal-treated cultures divided by counts per minute in control cultures. SI ≥ 3 is a positive response and SI ≥ 10 is a strongly positive response (shown in **bold**)

LTT-MELISA testing (Stejskal *et al.*, 1994). However, sensitization to several mercury compounds, as well as to other metals, is frequently observed.

Clinical observations accumulated over many years indicate that exposure to mercury can induce multiple sclerosis and other autoimmune diseases. As early as 1966, Baasch suggested that multiple sclerosis is caused by a neuroallergic reaction to mercury released from amalgam fillings, comparing it to an adult form of acrodynia (pink disease) (Baasch, 1966). Acrodynia occurred in some children who were treated with a mercury-containing teething powder (Warkany and Hubbard, 1953). The same conclusion – that dental and environmental exposure to mercury could be one of the factors leading to multiple sclerosis – was also reached by Ingalls (1983, 1986).

Recent research supports these early clinical observations. Prochazkova *et al.* (2004), at Charles University in Prague, studied the impact of amalgam replacement on health in patients with various autoimmune diseases who showed increased mercury-specific responses *in vitro*. After the replacement of mercury-containing amalgam with metal-free materials, 71% of the patients showed health improvement by 6 months later. In the group of patients that did not undergo dental treatment, no health improvement occurred.

Other studies seemingly contradict the hypothesis that mercury might be one of the causes of neurodegenerative diseases. Saxe *et al.* (1999) measured the concentration of mercury in the brains of Alzheimer's patients and controls. Since there were no statistically significant differences in brain mercury levels between the two groups, the authors concluded that mercury does not appear to be a neurotoxic factor in the pathogenesis of Alzheimer's disease. Similar findings were published by Clausen (1993), who studied mercury levels in the brains of patients with multiple sclerosis. The conclusions drawn from these studies may be questioned. In mercury-sensitized patients, even mercury concentrations within the normal range might provoke neuroallergic reactions in the brain.

The protocol of identification of metal hypersensitivity and removal of sensitizing metals has been successfully used in patients with fibromyalgia (Stejskal *et al.*, 2013) and autoimmune thyroid diseases (Sterzl *et al.*, 1999, 2006; Hybenova *et al.*, 2010). In the latter group, the removal of mercury-containing amalgam not only downregulated mercury-specific responses *in vitro*, but also resulted in a significant decrease of antithyroid peroxidase and antithyreoglobulin antibodies compared to levels prior to treatment.

Another disease of autoimmune origin is oral lichen planus. In one study, 72% of patients with oral lichen planus showed a positive response to mercury *in vitro* (Stejskal *et al.*, 1996). In addition to oral symptoms, the patients suffered from arthralgia, myalgia, eczema, and chronic ill health. After removal of amalgams, both local and systemic symptoms significantly decreased.

Finally, a study was recently published which showed successful treatment of orofacial granulomatosis on removal of amalgam in patients with a hypersensitivity to mercury (Tomka *et al.*, 2011).

Gold

The autoimmune potential of gold compounds has been known for many years. Serious side effects, such as nephropathy, were observed in some patients after the use of colloidal gold as a treatment for rheumatoid arthritis (Palosuo *et al.*, 1976), and the possible mechanisms behind these side effects have been discussed (Stejskal *et al.*, 1999). According to some studies, gold allergy is more common in patients who have developed autoimmune side effects after treatment with gold, indicating the existence of both allergy and autoimmunity induced by gold in the same patient (Möller *et al.*, 1996). It is important to emphasize that, as with other metals, gold allergy is not only expressed on the mucosa or skin, but also inside the body. For example, the rate of restenosis after implantation of gold-stented plates is high in patients suffering from gold allergy (Ekqvist *et al.*, 2007).

Nickel

Nickel is the most common sensitizer, and also the most studied (Thyssen and Menné, 2010). In Swedish patients with chronic fatigue syndrome (CFS), the frequency of nickel allergy was around 40%, as diagnosed by LTT-MELISA (Stejskal *et al.*, 1999). The coexistence of both allergic and autoimmune symptoms, induced by nickel, has been published, suggesting the autoimmune potential of nickel compounds (Kosboth *et al.*, 2007; Niedziela and Bluvshteyn-Walker, 2012). Direct evidence of nickel-induced autoimmunity was observed in susceptible rats that developed scleroderma-related autoantibodies and cutaneous sclerosis after exposure to nickel (Al-Mogairen *et al.*, 2010). Since nickel can also induce Toll-like receptors (TLRs) (Schmidt *et al.*, 2010), the autoimmune potential of this metal is plausible and should be studied in the future.

Aluminum

Aluminum is a ubiquitous metal, widely occurring in the environment and used in many everyday objects, foods, and pharmaceuticals. Aluminum is a well-known adjuvant in vaccines, despite its neurotoxic properties (Shaw and Tomljenovic, 2013). As described by Shoenfeld *et al.* (Shoenfeld and Agmon-Levin, 2011; Perricone *et al.*, 2013), adjuvants can promote ASIA in susceptible patients. Allergy to aluminum is rare, but has been described. Delayed-type hypersensitivity to aluminum and itching nodules were found in children exposed to aluminum-containing vaccines (Bergfors *et al.*, 2003). Exley *et al.* (2009) described a patient who developed CFS after multiple vaccinations with aluminum-containing

vaccines. A muscle biopsy confirmed the presence of aluminum-containing macrophages; the aluminum content in the patient's urine was also increased. Macrophagic myofasciitis (MMF) has been described by Gherardi and Authier (2012) as a systemic disease whose main histopathological feature is a granulomatous lesion comprising aluminum-loaded macrophages at the site of previous intramuscular vaccination. Typical clinical manifestations in MMF patients include myalgias, arthralgias, marked asthenia, weakness, cognitive dysfunction, and CFS. In addition, 15–20% of MMF patients may also have coexistent autoimmune diseases, the most frequent of which are multiple sclerosis, Hashimoto's thyroiditis, and diffuse autoimmune neuromuscular diseases, such as dermatomyositis, necrotizing autoimmune myopathy, myasthenia gravis, and inclusion body myositis (Authier *et al.*, 2001; Guis *et al.*, 2002).

Conclusions

Scientific literature and clinical experience show that metals play a key role in the development of autoimmune diseases. Whether metals induce autoimmunity or whether they aggravate existing disease, the removal of sensitizing metals upon identification of metal triggers has improved patient health.

Larger randomized studies in susceptible individuals, selected on the basis of genotypic or phenotypic biomarkers, should be pursued in the future. As suggested by Weiss and Liff (1983), studies of phenotypic markers may be suitable for the elucidation of causal pathways and identification of specific risk factors. The limited power of epidemiological studies to detect minor susceptible populations, such as those susceptible to mercury, has been discussed by Wallach *et al.* (2003). The benefits of this approach for patients can be monitored not only by the decrease in antibody titers (Sterzl *et al.*, 1999), but also by downregulation of metal-specific lymphocyte responses *in vitro* (Stejskal *et al.*, 1999, 2006, 2013; Yaqob *et al.*, 2006).

Finally, the identification of sensitized T cells in human blood can be made use of in future studies of vaccine-induced side effects. Elucidation of the possible mechanisms will contribute not only to successful treatment of affected individuals but also to the development of safer vaccines. The use of human blood lymphocytes in vaccine research has recently been suggested (Brookes *et al.*, 2014).

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