The role of environmental factors in autoimmune thyroiditis

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Abstract

Environmental factors can play an important role in the development of autoimmune thyroiditis (AT) and other autoimmune diseases. This article reviews the role of heavy metals and infectious agents in AT. Currently, the genes responsible for a metal-induced pathology are known in experimental animals but similar knowledge is lacking in man. Metals such as nickel or mercury induce delayed type T cell hypersensitivity (allergy) which is relatively common, especially in women. T-cell allergy can be studied with the lymphocyte transformation test, LTT-MELISA®. It has been found that patients with AT and other autoimmune diseases, such as multiple sclerosis, psoriasis, systemic lupus erythematosus and atopic eczema, show increased lymphocyte reactivity *in vitro* to inorganic mercury, nickel and other metals compared to healthy controls. The important source of mercury is dental amalgam. Replacement of amalgam in mercury-allergic subjects resulted in improvement of health in about 70% of patients. Several laboratory parameters such as mercury-specific lymphocyte responses *in vitro* and anti-thyroid autoantibodies were normalized as well. In contrast, no changes in health and laboratory results were observed in mercury-allergic patients who did not have their amalgams replaced. The same was true for non-allergic patients who underwent amalgam replacement.

Infectious agents such as *Helicobacter pylori* (*Hp*) may cause chronic inflammation and autoimmune reactivity in susceptible subjects. The results of *in vitro* experiments performed with lymphocytes from *Hp* infected patients indicate that *Hp* can cause immunosuppression which might be eliminated by successful eradication therapy.

In conclusion, heavy metals and *Hp* infection may play an important role in AT. Laboratory tests, such as LTT-MELISA® can help to determine the specific etiological agents causing inflammation in individual patients. The treatment of AT and other autoimmune diseases might be improved if such agents are eliminated and any future exposure restricted.

Abbreviations:

AT	- autoimmune thyroiditis
APS	- autoimmune polyglandular syndrome
PAA	- polyglandular activation of autoimmunity
HPA-axis	- hypothalamic-pituitary-adrenal axis
LTT	- lymphocyte transformation test
MELISA®	- Memory Lymphocyte Immuno Stimulation Assay
SI	- stimulation index
Hg-In	- inorganic mercury
Hg-O	- organic mercury
TPO	- thyroid peroxidase
Tg	- thyreoglobulin
Hp	- *Helicobacter pylori*
hHp	- heterologous *Helicobacter pylori* strain
aHp	- autologous *Helicobacter pylori* strain
HpAg	- *Helicobacter pylori* antigen
CagA	- cytotoxin associated gene A
PWM	- pokeweed mitogen

INTRODUCTION

Autoimmune thyroiditis (AT) is an organ-specific autoimmune disease and also the most frequently occurring autoimmune endocrinopathy. The etiology of autoimmune thyroiditis is multi-factorial. In addition to genetic predisposition, external factors such as physical and chemical influences as well as infectious agents can play an important role. A number of environmental factors have been postulated to be involved in the development of autoimmune thyroid diseases. This article will review the role of heavy metals and infectious agents.

In addition to the major form of AT, Hashimoto's thyroiditis, juvenile, postpartum, silent, atrophic and fibrous thyroiditis have been described (Volpé 1988). AT is characterized by the loss of thyroid cells and gradual destruction of the gland due to lymphocyte infiltration, leading to thyroid hormone deficiency. Patients can have symptoms of hypothyroidism at the beginning, sometimes of hyperthyroidism or euthyroidism, nodular or diffuse goiter, and ultrasonographic hypoechogenity. The presence of autoantibodies to thyroid peroxidase and thyroglobulin is an important diagnostic and predictive marker of the disease. Autoimmune reactivity can be caused by dysregulation due to imbalance of cytokines produced by Th1 and Th2 subtypes of lymphocytes. In Hashimoto thyroiditis, Th1 lymphocytes are predominantly stimulated and produce IL-2. IL-2 activates cytotoxic T cells which destroy target cells, thyrocytes (Volpé 1999). Also, inadequate function of regulatory T cells contributes to autoimmunity (Chatila 2005).

Another immunophathological mechanism involves antibodies against the TSH receptor. These antibodies are known to play a role in the development of another autoimmune thyroid disease, Graves' thyrotoxicosis (Sterzl 2006).

AT can occur as an isolated form, or as a part of autoimmune polyglandular syndrome (APS) or polyglandular activation of autoimmunity (PAA). In PAA, antibodies against other endocrine organs such as adrenal and reproductive organs are present, often without clinical symptoms of organ damage (Muir *et al.* 1994; Laureti *et al.* 1998; Sterzl *et al.* 1998; 1999a; 2007).

GENETIC SUSCEPTIBILITY AND HORMONAL INFLUENCES

In the etiology of AT, the genetic susceptibility, represented mainly by the antigens of the HLA system, such as HLA DR3 and DR4, is one of the most important factors. Recently, a specific amino acid signature of HLA-DR, which is strongly associated with AT, has been identified (Tomer *et al.* 2009). In addition, mutation of apoptotic genes Fas/FasL or polymorphism of CTLA-4, a major negative regulator of T-cell activation, may play a role in thyroid autoimmunity. Several other susceptibility genes have been described including CD40, protein tyrosine phosphatase-22, thyroglobulin and TSH receptor genes (Caturegli *et al.* 2007). Autoimmune thyroiditis, as well as other autoimmune diseases, occurs more often in patients with cellular immunodeficiency which might indicate genetic predisposition (Sterzl, 2006). Such patients may have a decreased ability to eliminate infectious agents and toxins. The agents trigger chronic inflammation and autoimmune reactivity. It is well known that allergic diseases such as asthma and atopic eczema are associated with AT (Guarneri & Benvenga 2007).

Autoimmune thyroiditis is more common in women and therefore, female sex hormones may play a role in the development of the disease. A significantly higher rate of monosomy X was determined in patients with autoimmune thyropathies (Inverzinni *et al.* 2005). Lastly, hormones such as cortisol and dehydroepiandrosteron and certain neurotransmitters might influence the balance of cytokines produced by Th1 and Th2 lymphocytes (Sterzl 2006).

AUTOIMMUNE THYROIDITIS AND ENVIRONMENTAL FACTORS

Deregulation of trace elements might be an important factor in the etiology of AT. As an example, a high iodine intake might enhance the antigenicity of thyroglobulin and lead to production of auto-antibodies. Ingestion of food depleted in selenium affects the activity of selenoproteins including glutathione peroxidases, and might result in a less efficient defense against heavy metals and other pollutants. Exposure to heavy metals, cigarette smoke, polychlorinated biphenyls, solvents, certain medicaments as well as stress and infections, might all be contributing factors (Duntas 2008).

Heavy metals such as mercury and nickel can participate in the development of AT by modification of autoantigens in genetically susceptible subjects (Stejskal & Stejskal 1999). The metal-driven inflammation may affect the hypothalamic-pituitary-adrenal axis (HPA-

axis) and trigger psychosomatic multi-symptoms such as chronic fatigue syndrome and fibromyalgia.

In rodents, mercury, gold and silver salts can either be tolerated or may induce a variety of skin and autoimmune diseases, all depending on the genotype (Bigazzi 1996). The situation is more complicated in humans where the genes responsible for metal-induced pathology are not known. In the absence of the knowledge of the susceptible genotypes, one can study patients with a known susceptible phenotype, such as patients with mercury allergy (Stejskal *et al.* 1996, Prochazkova *et al.* 2004; Sterzl *et al.* 2006a). Such patients have been identified by an abnormal lymphocyte proliferation to mercury and other metals *in vitro* (Stejskal *et al.* 1994, 1996; 1999) as well as by the presence of clinical metal allergy. Clinical metal allergy may be expressed as a skin reaction to nickel-containing items such as cheap earrings, jeans buttons, or as local or systemic symptoms experienced following dental treatment. Latter multi-symptoms such as fatigue and joint pain usually appear within 48 hrs following dental treatment and are due to inflammatory reactions induced by the release of metals ions (Stejskal *et al.* 2006, Yaqob *et al.* 2006).

Lymphocyte responses to heavy and transitional metals can be measured by LTT-MELISA®, which uses two independent determinations of lymphocyte proliferation after 5 day incubation with metal salts *in vitro* (Stejskal *et al.* 1994; Valentine-Thon & Schiwara 2003). This macro variant of the lymphocyte transformation test uses morphological identification of lymphoblasts as well as objective measurement of radioactive thymidine incorporation in stimulated lymphoblasts (Stejskal *et al.* 1994, 2006). The results are expressed as a Stimulation Index (SI), which is the ratio between ^{3}H-thymidine incorporated by metal-stimulated lymphocytes compared to non stimulated, control lymphocytes.

Sterzl and colleagues reported in 1999 (Sterzl *et al.* 1999b) that patients with AT coupled with chronic fatigue syndrome showed significantly increased lymphocyte reactivity *in vitro* to inorganic mercury and nickel as compared to healthy controls.

These findings have been followed by a study of the clinical relevance of metal allergy in patients with various autoimmune diseases including AT (Prochazkova *et al.* 2001, 2003).

In the studies of Prochazkova and colleagues mentioned above, all patients and healthy controls were exposed to metals (mercury, silver, tin, copper and zinc) present in dental amalgam on a long-term basis. The results show that lymphocyte proliferation to metals *in vitro* was significantly higher in the group of 42 patients with AT compared to 21 healthy controls. The most frequent allergens were nickel, inorganic mercury, tin, silver and cadmium (Figure 1a,b).

Bartova *et al.* (2003) showed that mercury from dental amalgam can be a risk factor in autoimmune diseases because it can induce the production of anti-nuclear autoantibodies in mercury-allergic patients with AT.

In the next study, the effect of amalgam removal on health of patients with various autoimmune diseases such as AT, systemic lupus erythematosus, multiple sclerosis as well as atopic eczema was studied (Prochazkova *et al.* 2004). The selection criteria for the inclusion of patients into the study was a positive lymphocyte response to low concentrations of inorganic mercury (0.5 µg of $HgCl_2$ per 1×10^6 lymphocytes per 1 ml culture and lower) and the presence of amalgam fillings in the oral cavity. Amalgams were replaced by composite materials following a protocol designed to protect the patients against metal dust generated during the procedure. Two weeks before and two weeks after amalgam removal, the patients were given 1 g of vitamin C and 50 µg selenium daily. Out of 35 patients diagnosed with autoimmune diseases including AT, a majority (71%) reported health improvement after amalgam replacement. This could also be demonstrated objectively by laboratory findings. The initial mercury-specific lymphocyte reactivity was significantly higher in the responder group that in those patients whose health did not improve after amalgam removal (29%). All patients with health improvement after amalgam replacement
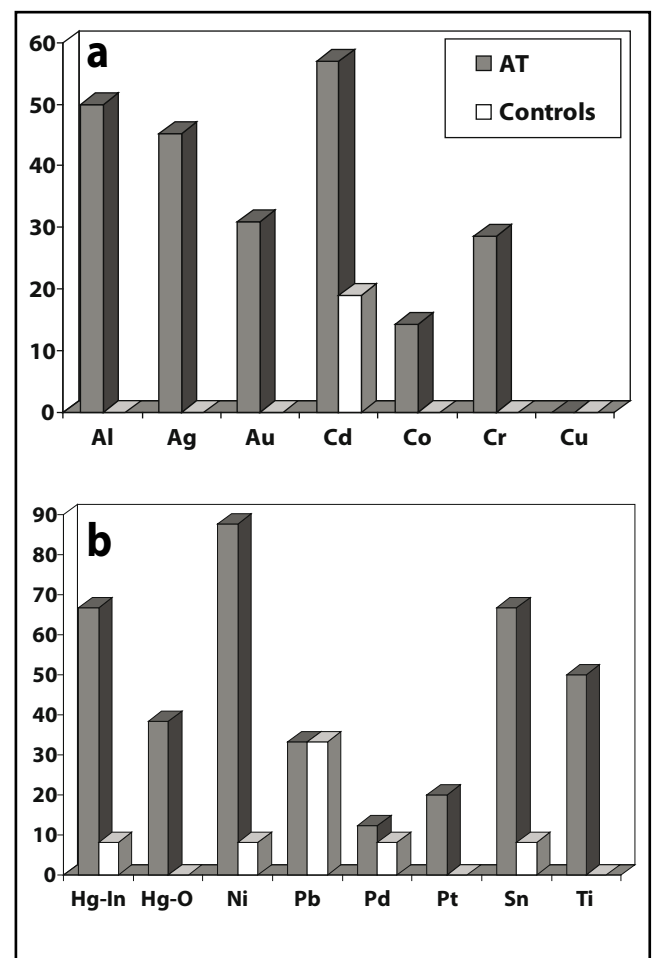

Fig. 1a,b. The percentage of positive responses to various metals *in vitro* in patients with AT and in healthy controls (Hg-In = Inorganic Hg, Hg-O = Organic Hg)

showed reduced proliferation to inorganic mercury *in vitro* in follow-up testing. A reason for why some patients' health did not improve after amalgam removal could be due to smoking. A few patients reported that after initially feeling better, they had started smoking again after amalgam replacement. Since these patients also reacted to nickel, it is possible that the exposure to nickel and mercury in cigarette smoke interfered with their general improvement of health.

In another study (Sterzl *et al.* 2006a), the influence of amalgam replacement on the levels of anti-thyroid antibodies in patients with AT was evaluated. From a group of 39 patients with AT, 27 patients responded to inorganic mercury *in vitro* while the remaining 12 did not and thus formed the control group. From the group of patients with mercury allergy, 15 patients underwent amalgam removal. Amalgams were not replaced in the patients in the control group. As in previous studies, amalgam was carefully replaced with composites. Anti-thyroid antibodies in serum were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Compared to levels at the beginning of the study, only patients with mercury allergy who underwent amalgam replacement showed a significant decrease in the levels of both anti-Tg ($p=0.001$) and anti-TPO ($p=0.0007$) autoantibodies. In contrast, the levels of autoantibodies in patients with or without mercury allergy who did not replace amalgam did not change (Figure 2a,b).
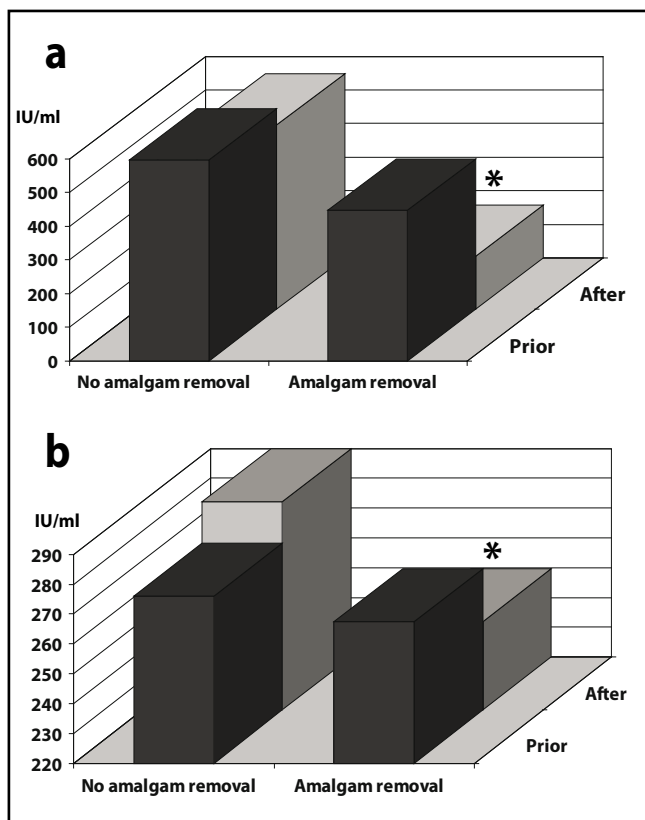

Fig. 2ab. Anti-TPO (2a) and anti-Tg (2b) antibodies in AT patients (IU= international units) *$p<0.05$

The studies described above indicate that the removal of amalgam in patients with mercury allergy may be a beneficial complement in the treatment of AT and other autoimmune diseases. This conclusion is in agreement with a policy of United States Food and Drug Administration which highlights the risk of amalgam in patients with a demonstrated or suspected mercury allergy. Memory T lymphocytes were found to be a useful biomarker for the identification of allergy-causing dental materials in individual patients as well as for monitoring of the decrease of metal-induced inflammation following avoidance of the allergen (Venclikova *et al.* 2006, 2007, Valentine-Thon *et al.* 2006, Stejskal *et al.* 2006, Yacob *et al.* 2006).

AUTOIMMUNE THYROIDITIS AND INFECTION

One of the etiological factors often discussed in connection to AT are infectious agents. For example, the *Yersinia enterocolitica* infection has been shown in patients with Graves's thyrotoxicosis (Bech *et al.* 1974; Bech *et al.* 1977; Heyma *et al.* 1986). *Helicobacter pylori (Hp)* seems to be another important causal factor. The *Hp* infection is ubiquitous and affects half of the world's population. *Hp* is a gram-negative, microaerophilic, spiral-shaped bacterium which causes persistent colonization of the gastric musoca with the development of serious gastric diseases such as chronic gastritis, peptic ulcer disease, adenocarcinoma and MALT lymphoma (Suerbaum & Michetti 2002; Sanders & Peura 2002). Despite of a cellular and humoral immune response, the host organism is often not able to eliminate the *Hp* infection. The lymphocytes predominantly differentiate to Th1 subtypes which are associated with cytotoxic reaction responsible for damage of gastric mucosa. Long-lasting chronic infection can lead to autoimmune reactivity or carcinogenesis (Krejsek & Kopecky 2004).

Besides gastric disorders, the *Hp* etiology is discussed in connection with the development of different extra-gastric diseases such as vascular, skin and autoimmune diseases (Martin de Argila *et al.* 1995; Tsang & Lam 1999; Realdi *et al.* 1999; De Koster *et al.* 2000; Nilsson *et al.* 2005; Solnic *et al.* 2006). In several studies, increased prevalence of *Hp* infection in patients with AT was observed and confirmed not only by higher anti-*Hp* IgG levels but also by positive urease breath tests (De Luis *et al.* 1998). Further, a strong positive correlation between the titers of anti-TPO antibodies and anti-*Hp* IgG levels was demonstrated (Bertalot *et al.* 2004). It was shown that monoclonal antibodies against the specific *Hp* antigen CagA react with thyroid follicular cells and that cagA-positive *Hp* carries a gene for endogenous peroxidase (Figura *et al.* 1999). The cross-reaction between antigens of the Lewis blood groups, Lewis X and Y, which are expressed on *Hp* lipopolysaccharide, gastric epithelium (on membrane H+, K+ ATP-ase pump), and also on the thyroid gland, was observed
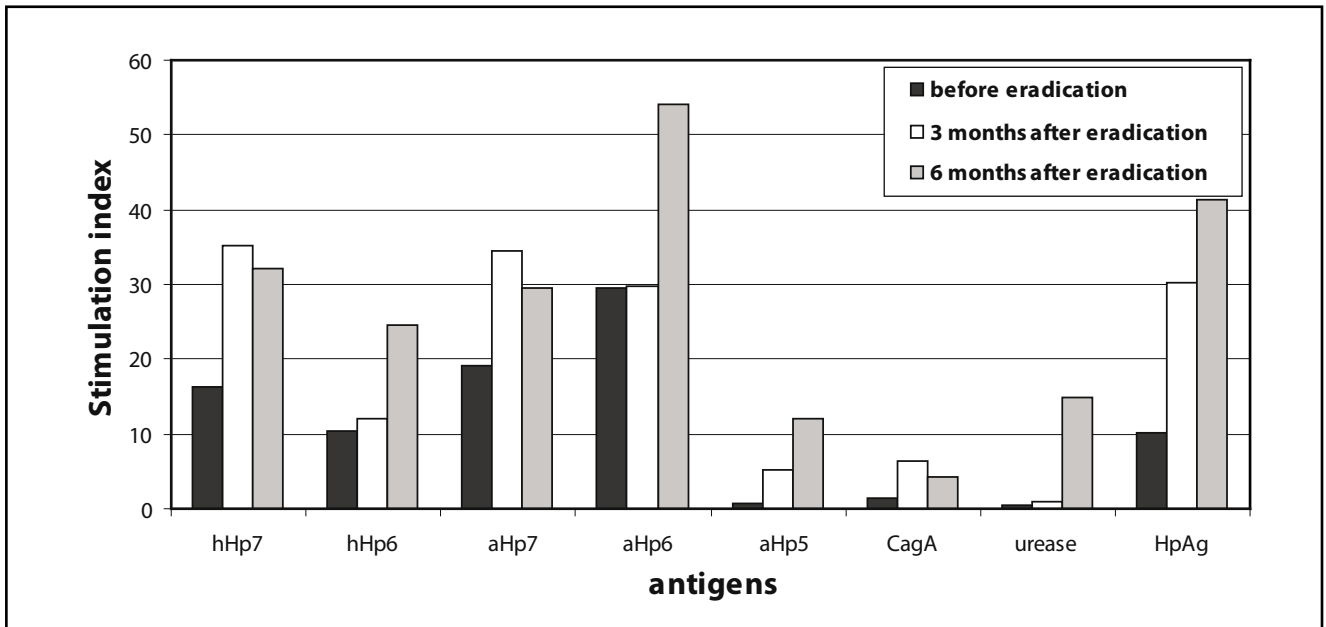
Fig. 3. Lymphocyte proliferation to various *Helicobacter pylori* antigens in patient with AT and *Hp* positive antral gastritis. Lymphocyte proliferation in PWM cultures: SI = 209 (prior eradication), SI = 236 (three months after eradication) and SI = 770 (six months after eradication). hHp = heterologous *Hp* strain, aHp = autologous *Hp* strain used in different concentrations 1×10^5, 10^6, 10^7 bacteria/ml. CagA, urease, HpAg = *Hp* antigen are commercial *Hp* antigens.

(Bertalot *et al.* 2004; Krejsek & Kopecky 2004). Thus, it is possible that the mechanism of molecular mimicry, a structural or sequential similarity, between the *Hp* and the host, may be one of the pathologic mechanisms in AT (Tomer *et al.* 1993).

Frequently, lymphoid follicles have been found in the gastric mucosa of young patients with AT (Cammarota *et al.* 1997). Larizza *et al.* (2006) described an interaction between HLA antigens DRB1*0301 and *Hp* infection in patients with AT and suggested eradication of *Hp* infection in children with AT and susceptible HLA alleles.

In 2006, Sterzl and colleagues have demonstrated the relationship between *Hp* infection and AT in three different groups of patients; patients with an isolated form of AT, patients with AT within APS, and those with AT as a part of PAA. In these three groups, different expressions of *Hp* antigens were observed. One important finding was that the immune response to *Hp* has been more prevalent in a genetically determined group of patients with isolated AT than in AT as a part of APS or PAA (Sterzl *et al.* 2006b). In addition, different genotypes of *Hp* were identified in different locations with pathological manifestations such as gastritis, tonsil cancer and AT (Pavlik *et al.* 2007). In patients with AT and *Hp* infection, gastric parietal cells antibodies were often found (Sterzl *et al.* 2008).

Another way to study the role of infectious agents in AT is to study T cell reactivity to specific agents *in vitro*. LTT-MELISA® has previously been used for the diagno-sis of acute borrelia infection and is helpful in clinically and serologically ambiguous cases of borreliosis (Valentine-Thon *et al.* 2007). We have measured specific cellular immune responses to *Hp* antigens in patients with AT and verified *Hp* infection. The laboratory testing was performed prior and after eradication therapy. In some patients with AT and *Hp* infection, lymphocyte responses to *Hp* as well as to the non-specific mitogen, PWM, were low. After successful eradication of the *Hp* infection, increased cellular reactivity to *Hp* as well as PWM was found (Figure 3). These preliminary data indicate that in some patients, *Hp* can cause immunosuppression which can be reversed by successful therapy.

CONCLUSION

Environmental factors such as heavy metals and *Hp* play an important role in the etiology of AT and other autoimmune diseases. Memory T lymphocytes can be used as biomarkers of susceptibility to mercury and other inflammation triggers in individual patients. If metal allergy is found, the patient should avoid all exposure to the allergenic substance. Mercury-allergic patients may benefit from replacement of dental amalgam. The treatment should be carried out in such a way as to minimize the exposure to mercury. Preliminary studies indicate that in some patients, *Hp* might cause immunosuppression which can be reversed by successful eradication therapy.

ACKNOWLEDGEMENT

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