

Autoimmunity and the environment

How can a chemical element elicit complex immunopathology? Lessons from mercury-induced autoimmunity

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Although most autoimmune diseases develop without a manifest cause, epidemiological studies indicate that external factors play an important role in triggering or aggravating autoimmune processes in genetically predisposed individuals. Nevertheless, most autoimmune disease-promoting environmental agents are unknown because their relationships to immune function are not understood. Thus, the study of animal models of chemically-induced autoimmunity should shed light on the pathways involved and allow us to identify these agents. The rodent model of heavy metal-induced autoimmunity is one of the most intriguing experimental systems available to address such questions. Although the ultimate pathophysiology of this model remains mysterious, recent studies have started to elucidate the mechanisms by which heavy metal exposure leads to immune activation and loss of self-tolerance.

Heavy metals and autoimmune disease

The list of chemical agents capable of producing or exacerbating autoimmune manifestations in susceptible individuals is constantly growing and includes hundreds of pharmacologic agents, hydrocarbon pollutants, pesticides, dietary supplements, and heavy metals. Reports of heavy metal-induced autoimmunity in humans including gold-, cadmium- and mercury-induced glomerulonephritis began to emerge over four decades ago [1–3]. These observations are summarized in Table 1. In particular, the abundance of mercury in the environment following natural degassing from the Earth's crust makes exposure unavoidable [4]. In addition there are a number of important anthropogenic sources of mercury which range from the mining industry to food products, to dental amalgams and energy saving light bulbs [5]. Metals other than mercury have also been implicated in the development of autoimmune disease (see textbox), but this review will focus on mercury since the animal model of mercury-induced autoimmunity (HgIA) has been by far the most widely studied example of heavy metal exposure. The neurotoxicity and nephrotoxicity of mercury have already been extensively described therefore this review will address only the autoimmune manifestations related to mercury exposure.

In the 1960s it was noted that mercury applied topically for the treatment of psoriasis lead to nephrotic syndrome [6], and a similar pathology was observed in women using mercury-containing skin-lightening cream shortly thereafter [1]. Many countries restrict the sale of creams containing this heavy metal, but their use leading to membranous nephropathy and the related minimal change disease, both associated with immunoglobulin and complement deposits in the glomeruli, is still reported in some areas of the world [7,8]. A retrospective study published in 2004 concluded that a positive correlation exists between exposure to mercury and the autoimmune necrotizing vasculitis Wegener's Granulomatosis [9]. The proliferation of T lymphocytes and the development of anti-laminin autoantibodies, a specificity mostly associated with the autoimmune disease pemphigoid, have been observed in individuals exposed to mercury vapors as an occupational hazard [10,11]. Additionally, urinary mercury levels of scleroderma patients correlate with disease severity and levels of anti-fibrillar autoantibodies [12]. The anti-fibrillar response seen in this subset of scleroderma patients is also found in the murine model of mercury-induced autoimmunity (HgIA) and susceptibility maps to major histocompatibility complex (MHC) class II genes in both humans and rodents [13,14]. It is noteworthy that gold and silver can also induce anti-fibrillar autoantibodies; here again, a strong association with particular MHC class II haplotypes has been described [15–17].

Another prominent source of human heavy metal exposure is dental amalgam, which continuously releases small amounts of mercury vapor. The safety of mercury-containing dental amalgams remains a controversial topic. The lack of any disease manifestations in the vast majority of individuals with mercury-containing amalgam argues strongly in favor of its continued use, nevertheless reports suggesting a role for this product in the onset of diseases such as multiple sclerosis (MS) have been published [18]. Indeed, clinical improvements in patients with systemic lupus erythematosus (SLE), autoimmune thyroiditis, or MS upon removal of mercury-based amalgam have been reported [19]. Similarly, some data has indicated a significant correlation between mercury exposure in dental workers and SLE, but the prevalence of these exposures was very low and thus these estimates are based on a small

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Table 1. Heavy metals which have been shown to promote autoimmunity in humans.

Source	Findings	References
Hg-containing skin creams	Case reports of membranous nephropathy and minimal change disease	[7,8]
Occupational and environmental Hg exposures	T-cell lymphoproliferation, anti-laminin and anti-nucleolar autoantibodies	[10,11]
Hg-containing dental amalgam	Controversial; a few animal and clinical studies support an association with autoimmunity	[2,18,19]
Gold chrysotherapy for rheumatoid arthritis	Autoimmune glomerulonephritis	[109]
Other	Hg associated with Wegener's granulomatosis; Hg levels in urine of scleroderma patients; Hg exposure and lupus	[9,12,20]

number of exposed cases and controls [20]. Furthermore, a controlled study has demonstrated that many symptoms attributed to dental amalgam are alleviated both by placebo and mercury chelation therapy [21], hence there is currently not enough systematic evidence to support the recommendation of amalgam removal in patients with autoimmune disease.

Rodent models of mercury-induced autoimmune disease (HgIA)

Disease course

HgIA has proven to be a very informative model for studying the effects of mercury exposure (see Table 2). The development of nephritis in Wistar rats injected with inorganic mercury (HgCl_2) led to the first report of HgIA in animals [22]. HgIA has since been induced not only by intraperitoneal and subcutaneous injection, but also by oral feeding and exposure to mercury vapor, making it a suitable model for mercury-induced autoimmune manifestations in humans who are exposed to mercury primarily via inhalation [23]. A reproducible membranous glomerulonephritis is induced by mercury in rats possessing MHC class II susceptibility alleles mapping to the RT-1 locus [24]. In male Brown Norway (BN) rats, HgCl_2 leads to polyclonal B and T cell activation, increased serum immunoglobulin levels, autoantibody production and glomerulonephritis with immune complex deposition [22,25,26]. The autoantibodies generated in this model are capable of binding phospholipids, DNA, glomerular basement membrane proteins, laminin-1 and thyroglobulin [27,28]. Within 4 to 5 weeks, manifestations of HgIA resolve in treated rats even when mercury dosing continues. Once the disease resolves, permanent resistance to subsequent mercury challenge is mediated by CD8^+ T cells, although the precise phenotype and mechanism of action of these T cells remain to be characterized [29–32].

In the murine model of HgIA, susceptible mice treated with HgCl_2 develop a T-helper 2 (Th2) biased polyclonal expansion of T and B cells accompanying a rise in serum IgG1 and IgE, the generation of highly specific IgG anti-fibrillar autoantibodies and glomerulonephritis of limited severity [33–36]. Anti-chromatin as well as anti-histone autoantibodies also appear in some mouse strains susceptible to HgIA [37]. Anti-fibrillar autoantibodies persist for months following withdrawal of mercury while the polyclonal activation and serum immunoglobulin levels decrease within 4 to 5 weeks. Unlike rats, mice do not develop resistance to mercury challenge at later time points [38].

In addition to causing autoimmunity *de novo*, mercury exposure also exacerbates other animal models of immune mediated disease. Lupus-prone strains such as (NZBxNZW) F_1 or MRL mice injected with HgCl_2 experience accelerated autoantibody production and renal immune complex deposits at a young age [39,40]. The graft versus host disease (GVHD) model of murine lupus is also accelerated by low-dose (20–200 $\mu\text{g}/\text{kg}$) HgCl_2 [41]. The methyl form of organic mercury (CH_3Hg) is also capable of exacerbating immune mediated disease. For instance, the severity of myocarditis induced by coxsackie virus B3 infection is increased following methyl mercury exposure, possibly via a mechanism involving inhibition of trace element binding to biological molecules [42,43].

The most prominent sources of human mercury exposure are mercury vapor released from amalgam tooth fillings, thimerosal (ethyl mercury) used in some countries as a vaccine preservative, and methyl mercury contamination of fish [4,5,44,45]. Organic mercury represents a major source of exposure in humans, but it is converted to inorganic forms *in vivo* [4]. Both organic and inorganic mercury can elicit HgIA in experimental animals, but the lower disease-inducing potential of organic mercury

Table 2. Experimental models of mercury-induced autoimmune disease .

	Rats	Mice
Genetic susceptibility	RT-1 locus of MHC class II - RT-1 ^l : Resistant - RT-1 ^{a,b,c,f,k} : Intermediate susceptibility - RT-1 ⁿ : Highly susceptible	I-A locus of MHC class II - H-2 ^a , H-2 ^b and H-2 ^d : Resistant - H-2 ^g and H-2 ^f : Intermediate susceptibility - H-2 ^s : Highly susceptible
Course	Self-limited disease resolving in 4-5 weeks	Polyclonal activation is self-limited and resolves in 4-5 weeks; anti-fibrillar response persists for months
Autoantibody specificity	DNA, phospholipids, glomerular basement membrane (GBM) proteins, laminin-1, thyroglobulin	Nucleolus (fibrillar)
Resistance following disease resolution	Yes	No

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suggests that it is the inorganic form (either directly administered or as a result of *in vivo* demethylation) that is responsible for autoimmunity. It is also difficult to draw clear comparisons between human and rodent dosages, as most human exposure is chronic, leading to accumulation over time [46]. Indeed, bioaccumulation of mercury in humans is affected by genetic factors, further complicating scale-modeling of dosages in model systems [47]. In the case of methyl mercury, polymorphisms related to the endogenous antioxidant glutathione (GSH) have been implicated in these inter-individual differences [48].

A spectrum of genetic susceptibility

In both mice and rats, the ability of mercury to induce autoimmune manifestations is dependent upon MHC class II susceptibility genes. As mentioned above, the RT-1 locus at least partially controls susceptibility in rats. While RT-1^l rats do not develop autoimmunity when treated with mercury, inbred rats with the RT-1ⁿ haplotype are susceptible to disease induction. Additionally, moderate susceptibility to HgIA is associated with RT-1a, b, c, f, and k haplotypes [49,50].

Genetic predisposition to mercury-induced autoantibody production in the mouse system maps to the I-A region of the

MHC class II locus [23]. The H-2^a, H-2^b and H-2^d I-A haplotypes render mice resistant to autoantibody production while H-2^s mice are highly susceptible. Intermediate susceptibility to the disease is observed in H-2^a and H-2^f expressing mice [37,51–53]. Studies aimed at characterizing the mechanisms by which the H-2 alleles control susceptibility to disease demonstrated that H-2^s x H-2^b F1 offspring co-dominantly expressing both I-A^s and I-A^b genes are resistant to mercury-induced autoantibody production, in contrast to other autoimmune diseases where MHC class II heterozygous expression increases or has little effect on disease manifestations [54–57]. It has been determined that resistance is due not to decreased levels of I-A^s, but to co-expression of I-A^b on B cells [54].

Mouse strains bearing the same H-2 haplotype display variable susceptibility to HgIA, for instance BALB/c, B10.D2, and DBA/2 mice all express H-2^d gene products, but differ widely in their reaction to mercury challenge, so clearly other non-MHC class II related genes are involved in disease induction. BALB/c display marked lymphoproliferation and immune complex deposition in glomeruli while B10.D2 mice display lymphoproliferation with very mild glomerulonephritis; DBA/2 mice are completely resistant [34,37,58].

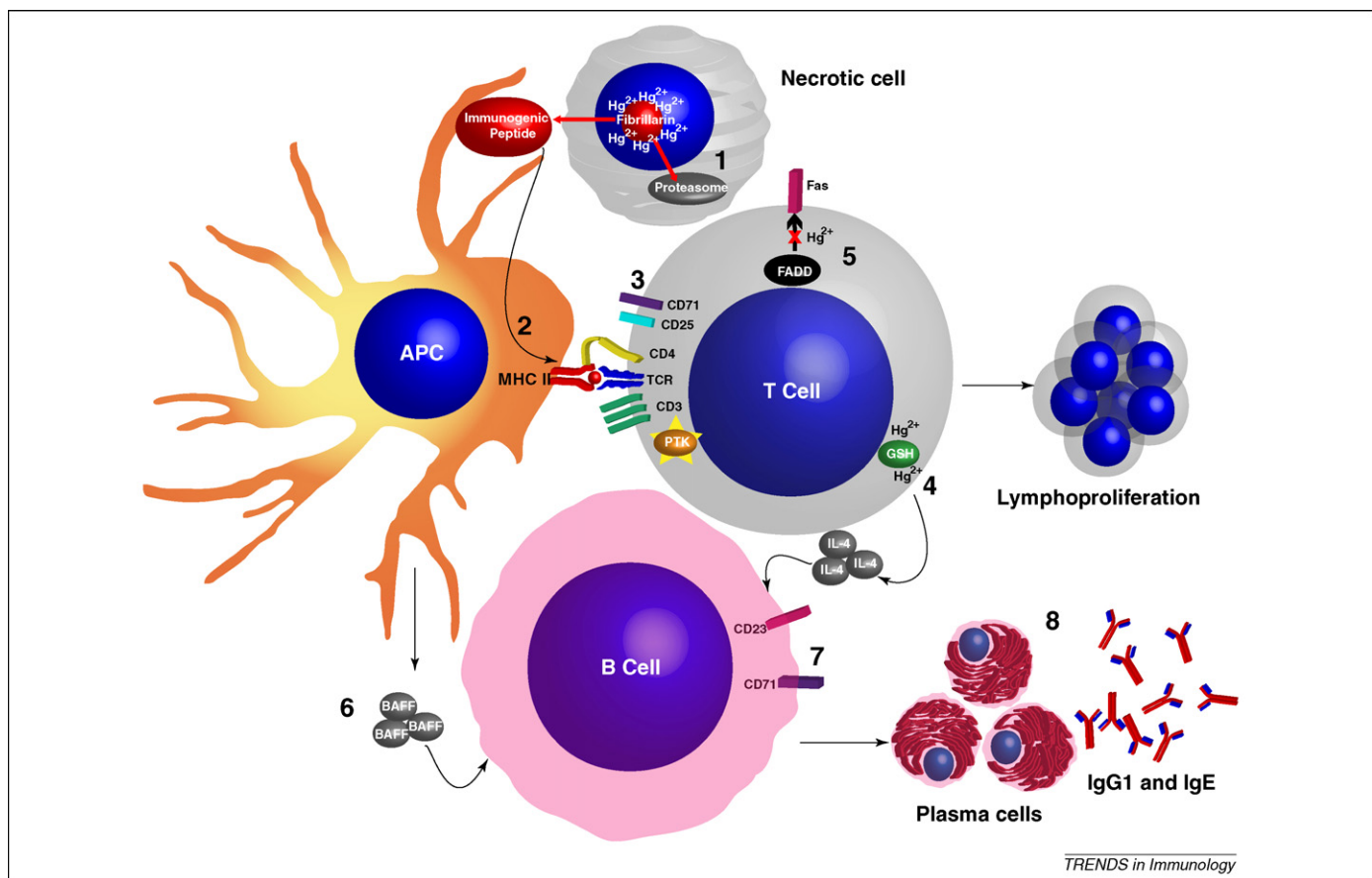


Figure 1. Putative mechanisms of mercury-induced autoimmunity.

(1) Mercury induces necrosis of somatic cells at the site of injection resulting in proteasomal processing of the nucleoprotein fibrillarin and its subsequent release as immunogenic peptides. (2) These peptides are then processed by antigen presenting cells (APCs) and presented to T cells in the context of MHC class II molecules. (3) Additionally, Hg results in the clustering of T cell receptors via a novel redox mechanism with subsequent protein tyrosine kinase (PTK) activation. The high affinity IL-2 receptor (CD25) and the transferrin receptor (CD71) are also upregulated by mercury. (4) Mercury binds to T cell intracellular proteins, affecting their function. Mercury binding to glutathione (GSH) promotes a Th2 phenotype resulting in the secretion of IL-4. (5) Recruitment of Fas-associated protein with a death domain (FADD) to the death-inducing signaling complex (DISC) during Fas signaling is also impaired, allowing escape of autoreactive T cells. (6) Serum B cell activation factor of the tumor necrosis family (BAFF) secretion by APCs is increased by mercury exposure and leads to proliferation of both T and B lymphocytes. (7) B cells are also polyclonally activated in the presence of mercury with upregulation of CD71 and CD23, differentiation into antibody-secreting plasma cells and consequently elevated serum immunoglobulin levels (8).

T cells in HgIA

Although no unifying molecular or cellular model exists to explain HgIA, several factors contributing to the disease process have been suggested and are summarized in Figure 1. Mercury ions exert effects on many biological molecules and their associated functional pathways via high-affinity interactions with amine, carboxyl, hydroxyl, and especially thiol groups [59,60]. For example, aggregation of cell surface receptors such as CD3, CD4 and other T cell receptor (TCR)-associated proteins can be promoted by mercury binding with resultant protein tyrosine kinase (PTK) activation and signal deregulation. A similar phenomenon has been reported for B cells [61,62].

The lymphoproliferation observed in the murine model of HgIA has been attributed to an expansion of T cells rather than B cells [63]. This study demonstrated that it is mature T cells that are capable of proliferation in response to HgCl₂ while thymocytes are not. This proliferation is under the control of non-MHC class II genes since BALB/c and DBA/2 mice both express H-2^d, but display opposing responses to mercury [64]. When splenocytes from the susceptible BALB/c strain were treated with mercury, both CD4⁺ and CD8⁺ subsets proliferated; only CD8⁺ cells proliferated in the disease resistant DBA/2 strain. The proliferative response of T cells to mercury actually appears to be TCR-dependent, although other factors such as antigen processing may be involved as well. Only cells bearing certain TCR V β chains proliferate in response to mercury; in BALB/c mice these include V β 6, 8, 10 and 14, and in SJL mice V β 6, 7 and 14 [65]. Likewise, in humans, CD4⁺ T cells that proliferate in response to HgCl₂ are skewed towards V β 2 expression [66].

The importance of T cell proliferation in disease onset and course has been demonstrated by studies using BN rats deficient in T cells as well as athymic (lacking mature T cells) or T cell-depleted mice. HgIA does not develop in these rodents, providing evidence of a crucial role for T cells in the HgIA disease model [67]. Indeed, markers of T cell activation such as CD25 and CD71 are rapidly upregulated upon exposure to HgCl₂ [68]. Further evidence is provided by the transfer of T cells from HgCl₂ treated rats or mice to naïve animals which results in autoimmune manifestations in the recipients [69,70]. The specificity of autoreactive T cells in this model is not fully understood, but is directed towards not only fibrillar in but apparently other nucleolar molecules as well [70].

B cells in HgIA

The marked polyclonal increase in serum IgG1 and IgE along with the production of autoantibodies indicates a critical role for B cells in HgIA, but there is only limited evidence for a direct effect of HgCl₂ on these lymphocytes. It has been reported that B cell populations and immature thymocytes from mice do not expand *in vitro* when treated with HgCl₂, but in some mouse strains mercury treatment does trigger B cell proliferation *in vivo* [63,71]. This expansion follows a measurable upregulation of the proliferation marker CD71. In A.SW mice (the H-2^s strain exhibits the highest susceptibility to HgIA), there is also an increase in expression of the low-affinity IgE receptor (CD23) after 7-

14 days of mercury administration [71]. This is in agreement with observations of increased IL-4 reported in this model [68]. Upregulation of CD23 driven by IL-4 has also been measured in mercury treated rats [72].

Normal B cells require B cell activation factor of the tumor necrosis family (BAFF) for their maturation through the transitional stages following their exit from the bone marrow. BAFF is elevated in the sera and parenchyma of mice genetically susceptible to spontaneous systemic lupus erythematosus (SLE) [73]. Work in our lab has revealed that mercury treatment also leads to increased serum levels of BAFF, but only in susceptible A.SW mice, not in resistant C57BL/6 or DBA/2 mice [74]. Because BAFF inhibits programmed cell death and promotes B cell survival, this protein might be responsible for the rescue of anergic autoreactive B cells. This argument is supported by the observation that BAFF blockade reduces disease manifestations in the mouse model of HgIA [74].

Co-stimulatory molecules and cytokines in HgIA

Proliferation in response to mercury is dependent on co-stimulatory molecules such as CD40L, CD80 (B7.1) and CD86 (B7.2). When these molecules are blocked by antibodies, mercury-induced T cell proliferation is impaired [63]. The role of co-stimulatory pathways in mercury-induced disease development has also been extensively studied *in vivo*. Systemic blockade of B7-CD28 interactions abate the manifestations of HgIA [75]. Our own lab has reported that specific blockade of CD80 or CD86 has differing effects, with blockade of the former completely inhibiting the formation of antinucleolar antibodies whereas blocking the latter is only partially inhibitory [75]. The inducible costimulatory molecule (ICOS) also promotes HgIA and its blockade decreases manifestations of the disease [76].

Co-stimulatory receptors which function to downregulate immune responses also play a role in HgIA. For example, antibody stimulation of 4-1BB, a molecule belonging to the tumor necrosis factor receptor (TNFR) superfamily, downregulates HgIA disease manifestations significantly [77]. Similarly, blockade of signaling by the negative regulatory molecule CTLA-4 both increases disease severity in susceptible rodents and results in antinucleolar autoantibody production in genetically resistant DBA/2 mice [78].

Cytokine levels are both affected by mercury and necessary for its autoimmune inducing potential. Blockade of the pro-inflammatory cytokine IL-1 inhibits T cell proliferation in response to mercury [63]. In addition to T cell proliferation, the polarization towards Th1 or Th2 phenotypes also plays a role in disease course. An effort to address this issue demonstrated that although CD4⁺ T cells are activated in both resistant and susceptible strains of mice (B10.D2 and B10.S, respectively), cells from susceptible strains produced a greater proportion of the signature Th2 cytokine IL-4. In contrast, resistant strains produced more of the Th1 cytokine interferon gamma (IFN- γ) [79]. The results of a similar study using rats support this finding [80], as do the results of a study utilizing an antibody to the Th1 associated OX221 molecule which lead to the exacer-

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bation of disease manifestations in BN rats [81]. Interestingly, IFN- γ is required for HgIA in B10.S mice, while IL-4 knockouts on this background develop autoantibodies in response to mercury [82]. A role for mast cells in the strain-dependence and Th2 polarizing effect of HgIA was suggested by *in vitro* treatment with HgCl₂. Mast cells from BN rats produced IL-4 in response to HgCl₂ while those from resistant LEW rats did not [83].

The biologic events behind mercury-induced polyclonal T and B cell activation are poorly understood. The high affinity of the metal for thiol-containing molecules can alter the availability of such species to immune cells [84]. GSH, the most abundant source of intracellular thiols, imparts changes in patterns of cytokine expression. Cellular levels of GSH impact mercury uptake, accumulation and toxicity [85]. GSH is required for induction of IFN- γ by the T cell mitogen concanavalin A *in vivo* [86] and therefore suppression of IFN- γ production by mercury in susceptible rats might be due to interactions with GSH [87]. Some studies conclude that mercury actually increases GSH levels, possibly by free radical-induced synthesis [88] although mercury-induced decreases in GSH levels have also been demonstrated in lymphocytes and monocytes [89]. Depletion of GSH in mice leads to decreased IFN- γ and increased IL-4 production by *ex vivo* stimulated T cells isolated from these animals [86]. Mercury-modulated decreases in GSH might therefore contribute to the Th2 cytokine profile of the HgIA model.

Although there is a strong argument for the role of Th2 polarization in rendering rodents susceptible to HgIA, IL-4 knockout mice can still develop antinucleolar autoantibodies (IgG2a and IgG2b) in response to mercury. Therefore although IL-4 is not required for breakage of self-tolerance, it is required for immunoglobulin class switch to IgG1 and IgE [90]. In contrast, IFN- γ does play a role in breaking self-tolerance, a point demonstrated by the drastically reduced levels of antinucleolar autoantibodies in mercury-treated mice lacking the gene for this cytokine [82].

Loss of tolerance via modulation and induction of cell death

Tolerance to self antigens can be overcome in part by the effects of mercury on specific biologic processes functioning to limit autoimmune manifestations. Lymphocyte deletion via CD95 (also known as Fas)-mediated apoptosis is attenuated by concentrations of mercury known to induce proliferation of splenocytes from susceptible strains of mice (5–10 μ M). Aggregation of the CD95 receptor on the cell surface is not affected, but recruitment of the adaptor protein Fas-associated protein with a death domain (FADD) into the death-inducing signaling complex (DISC) is disrupted [91,92]. Such inhibition could prevent the proper removal of autoreactive T cells that normally occurs in peripheral lymphoid organs. In both mice and humans, improper CD95 signaling gives rise to autoimmune disorders with features of SLE and rheumatoid arthritis [93–95].

The reason for the highly specific response to fibrillaritin observed in HgIA has yet to be fully explained. Evidence has been offered suggesting that mercury directly targets

fibrillaritin revealing cryptic epitopes via chemical modification which are capable of activating T cells [70,96–98]. This may be in part due to co-localization of fibrillaritin with proteasomes in the presence of mercury which has also been demonstrated *in vitro* and *in vivo*; other nucleolar proteins did not traffic to the proteasome in this study. This was demonstrated using human HEp-2 (a HeLa cell derivative), NIH-3T3 fibroblasts, and mouse ET (thymic epithelial) cells as well as primary splenocytes from B10.S mice [97]. Cytotoxic levels of mercury (40 μ M) result in direct molecular modification of fibrillaritin giving rise to novel antigenic properties; this chemical conversion is dependent on cysteine residues in the protein [96]. A 19 kDa fragment of fibrillaritin is produced during mercury-induced death of macrophages but not during death induced by other stimuli; this novel fragment is immunogenic while intact fibrillaritin is not [99]. Although mercury-induced modifications to fibrillaritin structure and processing likely play a role in breaking tolerance to this self-molecule, the exact mechanism by which fibrillaritin is so exquisitely targeted remains to be characterized. For example, proteasomal processing of a self-protein presumably results in presentation by MHC class I molecules, but susceptibility to HgIA maps to the class II locus. Mechanisms by which cross-presentation of self antigens with MHC class II and exogenous antigens with MHC class I can occur have been proposed with heat shock proteins being implicated in these processes [100–104]. Mercury does increase the levels of heat shock proteins in treated cells [105–108], and it may be possible that modified fibrillaritin is recognized as foreign by antigen-presenting cells (APCs), processed via the exogenous pathway and presented in the context of MHC class II molecules.

Conclusions and future perspectives

The role of environmental factors in the onset, progression and modulation of autoimmune processes is now well established from a variety of epidemiological and experimental studies. Animal models of heavy metal-induced autoimmunity offer unique laboratory tools to reveal the mechanisms by which chemical agents can lead to autoimmunity. The major challenge in HgIA research remains to elucidate the biochemical and immunological pathways by which a simple element like mercury can elicit both profound immune system activation and loss of self-tolerance. Developing *in vitro* experimental systems that reliably mimic features of this model would represent a useful tool in helping us understand these mechanisms. T cell peptide epitopes have yet to be identified during HgIA and their characterization would also go a long way to explain the MHC restriction in this model. The lesser autoimmunity-inducing potential of the organic forms of mercury is also puzzling considering that these compounds have an increased capacity for diffusion. Elucidating these questions will be critical in clarifying the role of chemical factors in autoimmune processes.

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Text Box: Metals and autoimmunity.

Heavy metals other than mercury have also been implicated in the development of autoimmunity. Gold salts were widely utilized in the treatment of rheumatoid arthritis, but chrysotherapy, as it was known, can elicit secondary autoimmune syndromes. In mice, both gold and silver can elicit autoimmune manifestations that are similar to those observed in HgIA (i.e. antinucleolar antibodies, MHC genetic restriction). In contrast, lead does not induce autoimmunity in these same strains, although it may accelerate disease in lupus-prone mice, as does cadmium, another significant heavy metal pollutant. There is no clear evidence that other metals, such as nickel, zinc or copper, are associated with autoimmunity. The well-known metal allergies elicited by these elements, such as nickel dermatitis, are usually type IV hypersensitivity reactions which involve modifications of self-epitopes by the metal (i.e. haptensisation).

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