

Neuroimmunotoxicology: Humoral Assessment of Neurotoxicity and Autoimmune Mechanisms

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The interactions between the nervous and immune systems have been recognized in the development of neurodegenerative disease. This can be exploited through detection of the immune response to autoantigens in assessing the neurotoxicity of environmental chemicals. To test this hypothesis, the following questions were addressed. *a)* Are autoantibodies to nervous system (NS) antigens detected in populations exposed to environmental or occupational chemicals? In sera of male workers exposed to lead or mercury, autoantibodies, primarily IgG, to neuronal cytoskeletal proteins, neurofilaments (NFs), and myelin basic protein (MBP) were prevalent. These findings were confirmed in mice and rats exposed to either metal. *b)* Do autoantibodies to NS antigens relate to indices of exposure? In humans exposed to either metal, and similarly in exposed rats, titers of IgG against NFs and MBP significantly correlated with blood lead or urinary mercury, the typical indices of exposure. *c)* Do autoantibodies correlate with sensorimotor deficits? In workers exposed to lead or mercury, a significant correlation was observed between IgG titers and subclinical deficits. Doses of metals used in rat exposures were subclinical, suggesting that autoantibodies may be predictive of neurotoxicity. *d)* Is the detection indicative of nervous system pathology? In rats exposed to metals, histopathology indicated central nervous system (CNS) and peripheral nervous system (PNS) damage. In addition there was evidence of astrogliosis, which is indicative of neuronal damage in the CNS, and the presence of IgG concentrated along the blood-brain barrier, as indicated by immunostaining for antibodies. *e)* Are immune responses to NS antigens pathogenic? Immunoglobulin fractions from rat and human sera interfered with neuromuscular function. These studies suggest that the detection of autoantibodies to NS-specific antigens may be used to monitor the development of neurotoxicity to environmental chemicals and that immune mechanisms may be involved in the progression of neurodegeneration. **Key words:** autoantibodies, autoimmunity, biomarkers, heavy metals, neurodegeneration, neuropathology, risk assessment. — *Environ Health Perspect* 107(suppl 5):767–775 (1999).

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The inaccessibility of the nervous system has long posed a challenge to neuroscientists and in particular to neurotoxicologists. This inaccessibility has impeded the evaluation of cellular and molecular changes associated with the initial changes of neurotoxicity, prior to overt clinical deficits in live animals and humans. Current methods of assessing the development of and recovery from neurotoxic insult include behavioral, electrophysiologic, and brain imaging techniques (1,2). However, these evaluations require highly trained personnel, are costly, and do not identify specific cellular substrates or mechanisms. This imposes limitations to their utility in clinical diagnostics, risk assessment, and their applicability to populations in the exposure arena (e.g., agriculture, industry). Furthermore, the detection of deficits often does not occur in the absence of overt manifestations.

O'Callaghan (3,4) has proposed that neurotypic and gliotypic proteins be used to detect and characterize the cellular response to toxicant-induced injury. Alterations in nervous system-specific proteins; for example, glial fibrillary acidic protein (GFAP), the astrocytic intermediate filament, may be expressed at low exposure levels of neurotoxic metals and toluene (3,5–7) and other nervous system

insults. This reactive gliosis occurs secondary to neuronal insult and degeneration. Although direct measurement of proteins in the brain may provide valuable insights into the cellular targets of neurotoxicity in animal studies (3,8,9), they are not applicable to human populations. However, this evidence should prove invaluable in evaluating potential biomarkers of neurotoxicity.

We have proposed that exposure to neurotoxic agents and the attendant neurodegeneration with the liberation of neural proteins can induce an autoimmune response conveniently measurable in blood. This immune response is reflected in the production of serum autoantibodies against nervous system proteins and activation of lymphocytes. Surprisingly, few neurotoxicology studies have investigated the possible involvement of the immune system and autoimmunity as a marker or as an effector in the development of neurotoxicity.

Neurocytology, Immunology, and Immunoprivilege

It is well established that the nervous system comprises the heterogeneous cell populations. Neurons, the excitable elements of the nervous system, are considered synonymous

with nervous system function. These are the cells responsible for detecting intrinsic and extrinsic environmental stimuli (sensory function), communicating these stimuli for interpretation (brain and spinal cord function), and formulating appropriate responses to these stimuli (motor function). Despite their being the minority cells of the nervous system, neurons control and regulate body function, thus placing them in a unique position of vulnerability. Neuroglia, astrocytes, oligodendrocytes, Schwann cells, and microglia are the majority cells. Functionally they provide protection, influencing electrical conductive properties of the neuronal axons and dendrites. Astrocytes, Schwann cells, and central macrophages (microglia), however, may play a role in immune activation within the nervous system.

Astrocytes may play a role in the regulation of inflammation and immune responses in the central nervous system (10). Astrocytes release interleukin (IL)-1 in response to substance P stimulation (11) and produce tumor necrosis factor (TNF)- α in response to viral infection (12,13) and bacterial lipopolysaccharide (LPS) (14). Viral or LPS stimulation induce the gene expression of IL-1 α ; IL-1 β ; IL-6, and interferon (IFN)- γ by astrocytes (12). Astrocytes also secrete IL-3, which induces microglial proliferation (15,16), suggesting a cooperative role between astrocytes and microglia during cell injury. Astrocytes also produce complement factors C3 and B (17). Astrocytes not only produce cytokines and complement factors but also respond to cytokine stimulation. Human astrocytes proliferate in response to TNF- α . This is accompanied by downregulation of GFAP expression (18). Stimulation of murine and human astrocytes by

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IFN- γ enhances their expression of class II major histocompatibility complex (MHC) (19,20). Astrogliosis, the astrocytic proliferative response to neuronal injury, may be in response to IFN- γ (21), whereas it is down-regulated by IL-4 (22).

Although astrocytes may not act as the major antigen-presenting cells (APCs), microglia act as APCs in the brain. These are among the first cells to be recruited in response to injury (23). In turn, microglia may stimulate astrocytes following injury through the production of TNF- α , IL-1 β , and IL-6 (23,24–26). The proliferation and APC activity of microglia has been shown to be stimulated by IFN- γ , colony-stimulating factor of macrophages (16,27,28). Microglia express both MHC I and MHC II, indicating their immunocompetence (29). Expression of both MHC I and II suggests that microglia may stimulate both T-helper (Th) and cytotoxic T cells (30).

Similarly, in the peripheral nervous system (PNS), Schwann cells respond *in vivo* and *in vitro* to IFN- γ and TNF and express MHC I and II (31,32). This ability of myelinating Schwann cells to act as APCs has been documented following peripheral nerve crush and in peripheral nerve disease (33,34).

Under normal circumstances, the nervous systems, both central and peripheral, are considered relatively immunoprivileged, as provided by the blood–brain barrier (BBB) and blood–nerve barrier (BNB). The BBB consists of specialized endothelial cells with tight junctions, pericytes, and bone marrow-derived perivascular elements. These are enclosed within a basal lamina and astrocyte foot processes (35). On the other hand, the BNB is formed by the permeable epineurium, the tight junctions of the perineurium, and the impermeable endoneurium (35). These barriers control the selective entry of essential biomolecules, including glucose, and exclude potentially harmful elements, including immune cells. However, this immunoprivileged status may be lost as a result of nervous system injury.

Interactions between Nervous and Immune Systems

As mentioned above, evidence has accumulated that glial cells (astrocytes, microglia, and Schwann in the periphery) play more than structural roles in the nervous system (36,37). Can these cells, or their constituents activate immune cells? GFAP (38) and myelin basic protein (MBP) (39) are mitogenic to cultured lymphocytes, *in vitro*. MBP is also mitogenic to cultured glia and lymphocytes. This evidence suggests that nervous system proteins may activate lymphocyte following demyelination or glial cell damage. It has now become accepted that interactions

and similarities exist between the nervous and immune systems (10,40).

Consistent with the possible role of glial cells as APCs are reports of lymphocyte infiltration across the BBB following its disruption (41,42) or if frank damage is absent, by adhesion to endothelial cells of the BBB (43–45). This adhesion and infiltration is modulated by intercellular adhesion molecule-1 and by cytokines. The presence of T and B lymphocytes in nervous system tissues and cerebral spinal fluid (CSF) has been documented in humans (e.g., multiple sclerosis [MS]) and in experimental models of nervous system disease (46,47). Experimental evidence indicates that activated T-lymphocytes are capable of entry into the central nervous system (CNS) without a need for antigen-specific activation (48), and that the CNS is constantly patrolled by a low but consistent number of activated T-lymphocytes and monocytes. This potential interaction between cells of the nervous and immune systems may have implications for the neurotoxicity of environmental and occupational toxicants.

Autoimmune Responses and Neurodegeneration

Autoimmune mechanisms have been recognized in neurologic diseases such as myasthenia gravis (49), Guillain-Barre syndrome (50), and experimental allergic encephalomyelitis (51). The nervous system itself may have immunologic functions as noted earlier (36,52). How nervous system antigens encounter and interact with cells of the systemic and lymphatic circulations and whether this occurs *in situ* in the nervous system or the periphery is not fully understood. This may occur in the brain parenchyma, in the cervical lymph nodes, excretion through the CSF, or at the BBB (53). The presence of nervous system antigens, GFAP and MBP in particular, has been documented in CSF of adults and children suffering from neurologic diseases, e.g., Alzheimer's disease and encephalitis (54–60). However, assay of these proteins in peripheral blood as markers of neurotoxic insult may be of limited use in the presence of extracellular proteases. On the other hand, autoantibodies against neurofilament triplet proteins (NFs) as well as MBP and GFAP have been detected in sera and CSF of human subjects suffering from neurologic disorders, including Alzheimer's disease, Parkinson dementia, amyotrophic lateral sclerosis (ALS), Creutzfeldt-Jakob disease, and kuru (61–67). Furthermore, autoantibodies to neurotypic proteins have been demonstrated in animal models of allergic encephalomyelitis and electroconvulsive shock (68,69). The interaction of environmental chemicals, neurodegeneration, and autoimmune responses has yet to be delineated.

Neurotoxic Exposures and Neuroimmune Interactions

As early as 1850, Aran (70) described a progressive muscular atrophy, today known as ALS or motor neuron disease. This was associated with occupational exposure to inorganic lead in some of his patients. Since then, several studies have reported similar observations with elevated levels of lead in plasma, erythrocytes, CSF, and spinal cords of ALS patients (71–74). Similarly, elevated mercury has been reported in patients with ALS (72,75,76). Heavy metals exposure has also been suspected of playing a role in the etiology of MS (77–80). In both ALS and MS, autoantibodies against nervous system proteins are detected. For example, autoantibodies against NFs, MBP, GFAP, and GM1 ganglioside have been detected in sera and CSF of human subjects suffering from these diseases (81–83). Anti-MBP antibodies are cytotoxic and are believed to play a key role in the pathogenesis of MS (65,81). More recently the presence of autoantibodies to the dihydropyridine calcium channel (the L channel) has been demonstrated in sera of ALS and Lambert-Eaton syndrome patients (82). These autoantibody titers correlated with disease progression. Such autoantibodies are pathogenic, interfering with nerve conduction, synaptogenesis, and neurite growth (83–85).

A role for environmental toxicants, particularly metals, in the development of nervous system pathologies and the possible involvement of the immune system are consistent with the experimental observations that metals, particularly lead, augment immune responses. For example, lead enhances B-lymphocyte differentiation *in vitro*. *In vivo* it enhances the activity of B lymphocytes toward T-cell–dependent antigens (86–89) and enhances the production of antibody *in vitro*, as well as directly activating B cells (90). Lead also enhances the production and release of IL-2 from T lymphocytes (91), thus enhancing B-cell responsiveness (90). These observations have led to the suggestion that lead exposure may result in an autoimmune response (90). Highly relevant to the presence of autoantibodies are studies indicating that low levels of lead enhance immunoglobulin synthesis (86,92). In the presence of chemical-induced nervous system degeneration and continued exposure to these chemicals, we believe that lymphocytes can be stimulated to increase autoantibody production. Several studies have demonstrated an increase in serum immunoglobulins following exposure to mercury and lead (93). This increase in serum immunoglobulins may reflect the appearance of autoantibodies.

Neuropathies and Cellular Antigens

Spencer and Schaumburg (94) and Chang (95) have suggested a unifying classification scheme for toxicant-induced neuropathy based on the primary site of insult and the subsequent secondary targets involved. These categories include the following: *a)* Neuronopathy, where the soma (cell body) is the primary target, followed by secondary nerve fiber degeneration (dying-forward), tertiary myelin loss, and target cell atrophy or death (trans-synaptic degeneration). Neuronopathies are irreversible since neurons are terminally differentiated. Agents such as mercury, aluminum, and glutamate-induced excitotoxicity are believed to precipitate this type of insult. *b)* Axonopathies, where the axons (or peripheral sensory fibers) primarily targeted are believed to be the most common nervous system injury, particularly the exposed vulnerable peripheral nerves. Axonopathies may progress proximo-distally from the soma or die-back, progressing to a neuronopathy. Secondarily, axonopathies are associated with secondary myelin degeneration in severe cases. Where the soma remains functionally intact, chromatolysis and fiber regeneration may occur. Toxicants inducing axonopathies include acrylamide, hexacarbons, and tri-*o*-cresyl phosphate. *c)* Myelinopathies, or myelin gliopathy, involve either the myelin sheath, such as in the case of triethyltin, or the myelinating cells, such as in the case of ethidium bromide neurotoxicity. This may or may not be followed by axonal degeneration. Less well classified is the damage that may occur to astrocytes. It is suggested that astrocytes may be primarily or secondarily targeted by some neurotoxic agents.

In the nervous system, astrocytes act as sites of deposition for heavy metals, including mercury (96,97) and lead (98,99), where these metals may modulate metabolic function (100,101). In this regard, astrocytes may themselves be targets of lead and mercury, resulting in their degeneration and death, whether as a primary target or secondary to astrogliosis (102–106). Similarly, other chemicals may induce damage to glia. Pilocarpine-induced epilepsy in rats resulted in glial damage within the substantia nigra and basal cortex (107). Recently, toluene inhalation has been reported to result in a reduction in the astrocyte-specific protein GFAP (7). Additionally, some chemicals may induce a vasculopathy, resulting in leakage of barriers. Inorganic lead and cadmium fall within this category (94).

On the basis of this classification, we propose that detection of immune responses to autoantigens characteristic of different target sites may be used in identifying the

effects and specific targets of the neurotoxic chemicals. Coupled with neuropathologic assessment, these autoimmune responses can be validated for experimental animal studies and the monitoring of human populations. A summary of potential cellular targets and cellular antigens in the nervous system is presented in Figure 1. Additional nervous system-specific proteins are discussed by O'Callaghan (4) and Linington and Brostoff (107).

Autoantibodies as Markers of Nervous System Injury: Rationale and Hypothesis

In our use of the term marker or biomarker, a distinction is made here between markers of exposure and markers of effect. For example, blood lead levels are used to indicate exposure to lead; they do not provide information on

the neurotoxicity of lead. It is inferred from historical human and animal studies that a particular blood level is associated with certain symptoms or lesion. Furthermore, this inference is often based on detection of overt toxicity but does not delineate changes that may be early predictors of toxicity. A second distinction is also made here. In the study of the link between environmental chemicals, autoimmunity, and the nervous system, we distinguish between the concept of immunologic biomarkers of neurotoxicity, immune mechanisms of neurotoxicity, and the immunotoxic effects of environmental chemicals. The latter is discussed in other reviews. The concept of using immunologic indicators to reflect environmental exposure-induced damage to other systems derives from the definition of the immune system as a

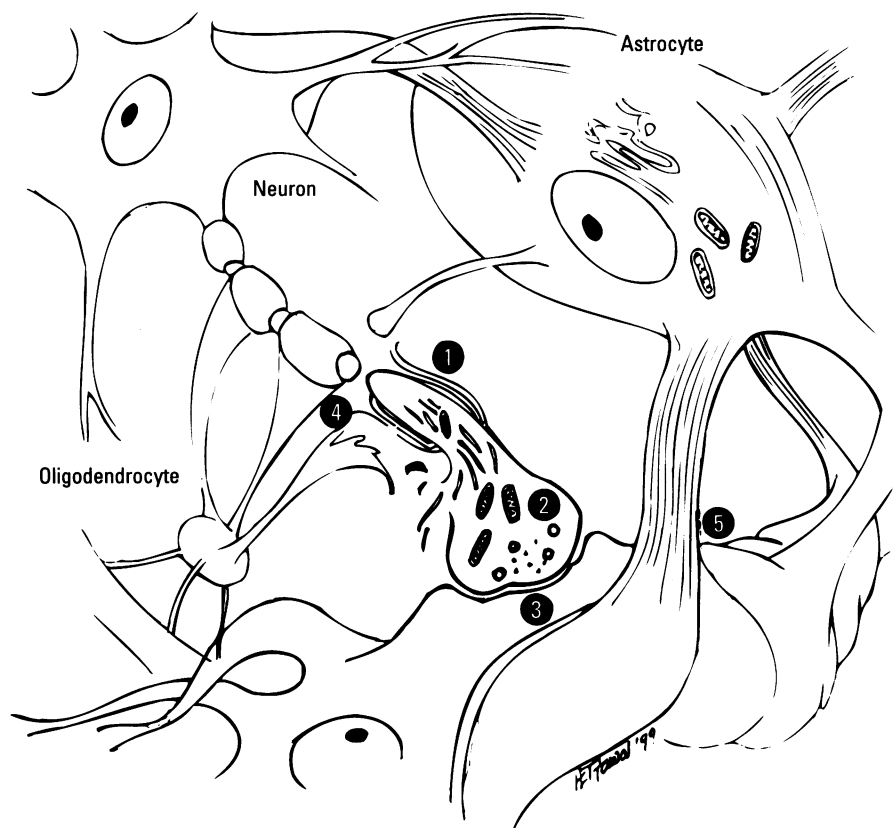


Figure 1. Potential targets and autoantigens to which autoantibodies may be detected as a result of neurotoxicity. Abbreviations: ChAT, choline acetyltransferase; CNS, central nervous system; MAPs, microtubule-associated proteins; MT, microtubule; NFs, neurofilament triplet proteins; PNS, peripheral nervous system. (1) Structural proteins of the neuron such as the NFs, α - and β -tubulin of the MT, and MAPs. (2) Proteins of neurotransmission such as sodium, potassium, and calcium channels or those associated with the vesicle-docking fusion essential for neurotransmitters release (e.g., synaptophysin, synapsin, synaptotagmin, latrotoxin receptor), or intracellular neurotransmitter synthesizing enzymes (e.g., ChAT). (3) Proteins of the postsynaptic targets: neurons/muscle (e.g., neurotransmitter receptors, actin, myosin, desmin) or catabolic enzymes (e.g., acetylcholinesterase). (4) Proteins of myelin and myelinating cells such as myelin basic protein and myelin-associated glycoprotein, common to both the CNS and the PNS, or proteolipid protein, found only in CNS myelin. P₀ and peripheral myelin protein-22 of peripheral myelin. (5) Proteins associated with astroglia of the CNS or the blood-brain barrier, such as glial fibrillary acidic protein, S-100, and vimentin (in developmental studies). Furthermore, although some neuronal proteins are widely distributed throughout the nervous system, others are restricted to only certain neurons or brain regions (G-substrate in the cerebellum, DARPP-32 in the neostriatum, and SNAP-25 in the hippocampus). Proteins of the CNS and PNS are reviewed in (4) and (107).

functional system responsible for surveillance of the host's various organ systems. The immune system provides a unique opportunity for the neurotoxicologist, particularly in the reduction in the number of experimental animals and in clinical toxicology, for assessing the pathologic status of other systems. This is facilitated by the relatively noninvasive sampling and collection of sera and immune cells. Although this approach has been used successfully by toxicologists in the study of other systems [reviewed in (108–112)], a review of the literature reveals a paucity of studies dealing with neurotoxic exposures and autoimmune responses (113).

The generation of autoantibodies to nervous system-specific antigens is feasible in the presence of neuronal death, axonal degeneration, demyelination, and glial death such as that precipitated by neurotoxic agents in the CNS or PNS. This exposes and liberates intracellular antigens, often sequestered antigens that the immune system has not encountered previously. Our hypothesis is summarized in Figure 2. Proteins specific to nervous system structures would be presented as autoantigens by resident microglia in the CNS or as infiltrating macrophages in the PNS. This would result in a cellular and/or humoral response with autoantibodies raised against these autoantigens in the latter case. The generation of these autoantibodies would likely occur, particularly with metal intoxication, in light of their ability to compromise blood barriers (103,104,114,115). In this way, the immune system provides a means whereby cellular damage in the nervous system can be documented and measured in serum, thereby eliminating the problems associated with the inaccessibility of the nervous system.

Autoantibodies in Humans and Animals Exposed to Neurotoxic Agents

The use of autoantibody detection to indicate nervous system insult was tested in our laboratory in several populations and verified in animal studies (Table 1). To illustrate the utility of these assays, we will concentrate on results obtained from inorganic lead exposures.

Autoantibodies and Exposure

Field testing of autoantibody assays was performed by our laboratory in male workers occupationally exposed to lead at a battery factory (blood lead: 10–40 µg/dL) or mercury vapor (urinary mercury: 6–30 µg/g creatine) at a fluorescent light factory. In addition, a reference group from a frozen food packing plant with no prior work history of exposure to either metal was also recruited (116–118). All participants in the study were matched on the basis of demographics and socioeconomic

status as well as on years of metal exposure. Ambient mercury (as vapor) and lead (as dust or fumes), 0.05 and 0.09 mg/m³, respectively, are below or at the threshold limit value–time weighted average (TLV-TWA)

adopted by the American Conference of Governmental Industrial Hygienists.

Titers of autoantibodies (IgM and IgG isotypes) to NF proteins NF-68, NF-160, and NF-200; GFAP and MBPs were determined

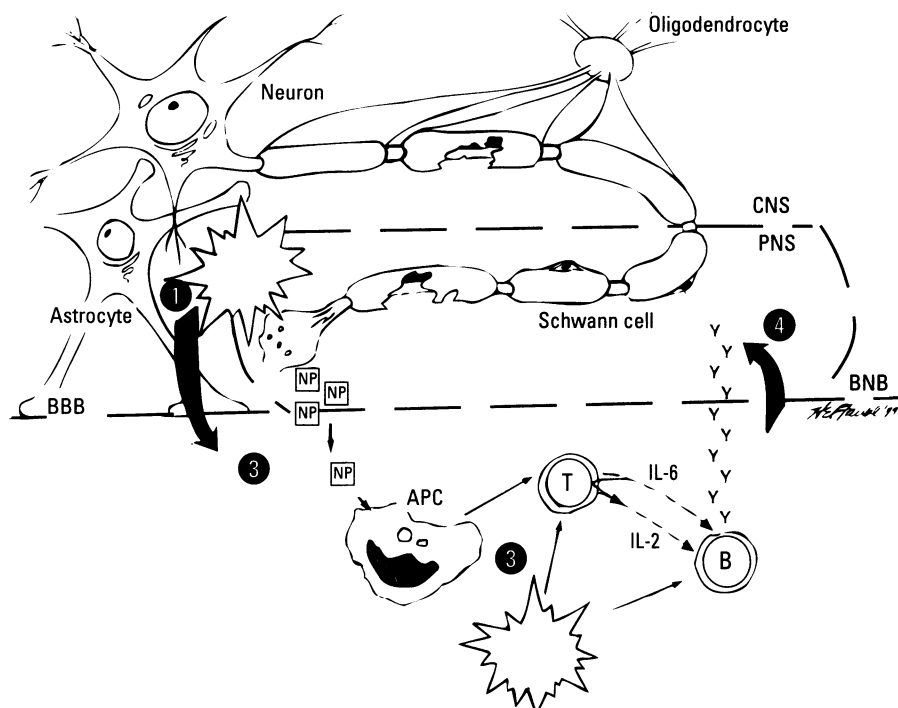


Figure 2. Hypothesis for the induction and pathogenesis of autoantibodies to nervous system proteins during exposure to environmental chemicals. Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; APC, antigen-presenting cells; B, B lymphocyte; BBB, blood–brain barrier; BNB, blood–nerve barrier; CNS, central nervous system; IL, interleukin; NP, nervous system protein; PNS, peripheral nervous system; T, T lymphocyte; Y, antibody. Following the initial insult (1) by neurotoxic chemical to cells of the nervous system, autoantigens ([NP] see Figure 1) are released and (2) undergo antigen processing and presentation by microglia, or astrocytes or macrophages. (3) This results in T-lymphocyte activation and stimulation of B lymphocytes by interleukin signaling (e.g., IL-6 and IL-2) to produce autoantibodies to these autoantigens. This may occur *in situ* within the nervous system or as a result of being extruded into the peripheral circulation. The perception that these antigens are foreign is facilitated by the presence of BBB and BNB. In addition, environmental chemicals may act directly on the immune system to augment the development of cellular or humoral responses. (4) Autoantibodies (Y) may be pathogenic, contributing to the progression of the neuropathology or physiologic impairment. Autoantibodies may directly induce damage to neurons or compromise function by complexing to surface antigens, fix-complement to precipitate neuronal death, or damage neural tissue by ADCC.

Table 1. Chemical exposures where autoantibodies to nervous system proteins are detected.

Exposure	Medium	Species	Reference
Phenyl saligenin phosphate (po)	Serum	Chicken	(155,156)
Lead acetate in drinking water	Serum	CBA/J mice	(123)
Lead acetate in drinking water	Serum	ALAD mice	(165,166)
Lead acetate in drinking water	Serum	Fischer 344	(120,133)
Trimethyl lead	Serum	Fischer 344	(168)
Methyl mercury in drinking water	Serum	Fischer 344	(121)
Cadmium chloride (po)	Serum	Fischer 344	(171)
Trimethyl tin (ip)	Serum	Long Evans	(171)
Cobalt	Serum	Sprague-Dawley	(170)
Trimethyl lead in food	CSF	Macaque	(171)
Toluene by inhalation	Serum	Macaque	(171)
Cadmium in battery plant	Plasma	Human	(172)
Lead in battery plant	Serum	Human	(113,116,117)
Mercury in fluorescent light plant	Serum	Human	(113,118)
Mercury (amalgam)	Serum	Human	(170)
Trichloroethylene and solvents	Serum	Human	(108)
Methamidophos	Serum	Human	(173)
Pesticide mixtures	Serum	Human	(174)

Abbreviations: CSF, cerebral spinal fluid; ip, intraperitoneal; po, per os.

in sera of exposed and reference populations by an enzyme-linked immunosorbent assay developed by El-Fawal et al. (119) against these proteins. Summaries of individual titers, the mean, and the percent of each population with detectable immunoglobulins (M and G isotypes) against NF-68 in all three populations are presented in Figure 3. Autoantibodies against neuroproteins predominated in metal-exposed populations compared to the reference population. The detection of autoantibodies in a small percentage of the reference population probably reflects natural autoantibodies, usually IgM, which are found in some individuals (63,120).

The generation of autoantibodies to these antigens during lead or mercury exposure was

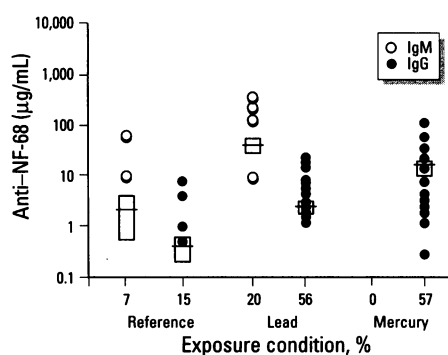


Figure 3. Individual autoantibodies (IgM and IgG) titers to NF68 in reference population and populations exposed to lead at a battery plant or mercury in a fluorescent light factory. NF, neurofilament. The y -axis is a \log_{10} scale. Percentage positive values for anti-NF-68 are indicated below the x -axis. Horizontal lines and boxes indicate means \pm SEM. Autoantibody detection predominated in exposed populations compared to the reference group. The prevailing isotype in exposed populations was IgG (see text).

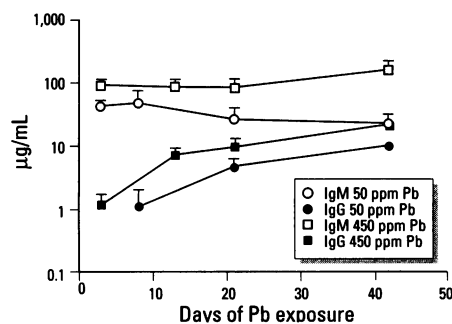


Figure 4. Autoantibody (IgM and IgG) titers to NF-68 in rats exposed to lead acetate at 50 and 450 ppm in the drinking water. Abbreviations: Pb, lead; NF, neurofilament. Control rats did not have detectable titers of autoantibodies to the assayed antigens. The y -axis is a \log_{10} scale. Each point represents the mean \pm SEM for 7 rats. Autoantibodies were detected as early as 4 days of exposure and were of the IgM isotype (primary response). IgG was detected later, as isotype switching occurs with no significant increases in IgM. Furthermore, titers were significantly higher in animals exposed to the higher dose (see text and Table 2).

confirmed (119,121). In studies with lead (121), male Fischer 344 rats (> 42 days of age) were exposed to 50 or 450 ppm lead acetate in the drinking water. No overt signs of toxicity or changes in home-cage behavior (122) were evident in any of the rats. Blood lead levels ranged from 11 to 50 $\mu\text{g}/\text{dL}$ and were comparable to those measured in humans. While control rats had no detectable titers to these particular antigens, titers of autoantibodies, particularly IgM, were detected and quantified as early as 4 days after the initiation of lead exposure. IgG was detected later. The detection of anti-NF-68 and the time course are summarized in Figure 4. The early detection of IgM is consistent with a primary antigen challenge (in this case autoantigens). There was significant elevation of IgM titers, followed by a gradual isotype switching to IgG at later durations. In both human and rat studies, autoantibody levels, particularly anti-NFs, correlated significantly with blood lead—the index of exposure for both humans and rats (Table 2).

To confirm the presence of autoreactive B lymphocytes expressing autoantibodies to nervous system proteins, splenocyte preparations from lead-exposed rats and mice (123) were challenged with LPS. Both IgM and IgG against NFs, GFAP, and MBP were detected in the splenic supernatants. Figure 5 shows the IgM response of LPS-stimulated rat splenic cells at 7 days of exposure. In mice the production of autoantibodies from splenocytes was accompanied by IL-6 and IL-2 production by T lymphocytes (concanavalin A-stimulated cultures). IL-6 is believed to stimulate B-lymphocyte differentiation to antibody-producing plasma cells and copromotes IL-2 production by mature T lymphocytes. IL-2 promotes secretion of antibodies from activated B lymphocytes and is consistent with Th-assisted B-cell responses (102).

Autoantibodies and Clinical Deficits

In exposed human populations, antibody titers that significantly correlated with exposure and sensorimotor function were predominantly IgG. IgG is the isotype most commonly associated with secondary antigen challenge or antigen persistence and pathology (124). A significant dose-response relationship between the total number of detectable autoantibodies of the G isotype (IgG score) to the five antigen

proteins and the total number of upper and lower limb sensorimotor deficits (clinical score) was observed (Figure 6). This suggests an association between the appearance of antibodies in the sera and the neurotoxicity of metal exposure. However, it must be emphasized that sensorimotor deficits were subclinical and not overt.

Antibodies and Cellular Targets

The neuropathology associated with chronic exposures to mercury or lead primarily involves the neuroaxon with secondary demyelination (105,115,125). In the human studies, anti-NFs, IgG isotype, were the most frequently detected antibodies. These were also the antibodies and the isotype best correlated with blood lead or urinary mercury and clinical scores of sensorimotor deficits. This is consistent with the type of neuropathy in humans that manifests primarily neuroaxonal damage. Furthermore, the detection of anti-GFAP titers in these studies supports the targeting of the CNS and astrocytes by heavy metals (96–98), since astrocytes are exclusively found in the CNS. In this manner, autoantibodies may provide a means whereby subcellular targets and the progression of neuropathy may be documented.

In this context the temporal appearance of autoantibodies in serum provides information on the extent of target cell involvement and the progression of insult. For example, it is likely that anti-NF titers would be the first to appear with primary neuronal involvement (e.g., mercury), whereas anti-MBP would precede all others in the case of primary demyelination (triethyltin).

Toxic Exposures May Precipitate Autoimmunity by Altering Antigen Immunogenicity

The role of environmental factors in the production or augmentation of autoimmune responses has been suggested for some time. The interaction of drugs or environmental chemicals, including metals (e.g., gold, mercury, and glomerulonephritis), with endogenous protein constituents may alter their immunogenicity and result in the induction of immune responses reflected in the generation of autoantibodies (108). Having detected

Table 2. Correlation (r) and significance level (p) between autoantibody titers and blood lead in workers occupationally exposed to lead and rats experimentally exposed to lead in the drinking water.

		Anti-NF-68	Anti-NF-160	Anti-NF-200	Anti-GFAP	Anti-MBP
Human PbB ($n = 50$)	$r =$	0.5	0.3	0.3	0.1	0.2
	$p =$	0.0001	0.002	0.02	NS	NS
Rat PbB ($n = 25$)	$r =$	0.7	0.5	0.7	0.4	0.3
	$p =$	0.0001	0.001	0.0001	0.05	0.05

Abbreviations: PbB, blood lead; NS, not statistically significant.

antibodies to nervous system antigens in humans and experimental animals, it was speculated that further alteration of these antigens may occur with chronic exposures to neurotoxicants. This may provide a basis for differential antibody profiles between chemicals. Waterman et al. (123,126) have demonstrated that *in vitro* treatment of the neural antigens NFs, MBP, and GFAP with lead, followed by inoculation of CBA/J mice, resulted in antibody titers that were significantly higher than those produced by the

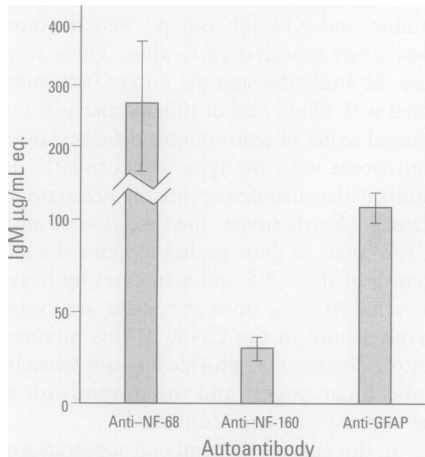


Figure 5. IgM in splenic supernatant following stimulation of rat ($n = 7$) splenocytes collected from rats 7 days after exposure to 450 ppm lead acetate in the drinking water. Abbreviations: GFAP, glial fibrillary acidic protein; NF, neurofilaments. Splenocytes were cultured for 72 hr with lipopolysaccharide in M199 supplemented with 5% fetal bovine serum. Cell-free supernatants were collected and assayed for autoantibodies to nervous system proteins. For further details see Waterman et al. (126). This verifies the presence of an autoreactive B-lymphocyte population to nervous system proteins. Control splenic supernatants did not have detectable titers of antibodies to these proteins (see text). Each bar represents the mean \pm SD.

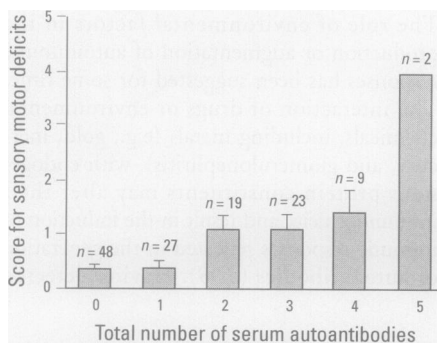


Figure 6. Dose-response relationship between clinical scores and the number of detectable IgG titers against nervous system proteins in reference, lead-exposed, and mercury-exposed humans ($n = 129$). These data indicate that as more autoantibodies against nervous system proteins are detected the greater the likelihood of clinical deficits due to greater neurotoxicity, $p < 0.0001$ (see text). Each bar represents the mean \pm SD.

antigens alone. MBP is a negatively charged 18-KD protein with 170 amino acid residues with no sulfhydryl groups (127). Lead cations, which are divalent, may bind to two MBP molecules in a charge neutralization process (126) to produce a larger, more immunogenic antigen. In contrast, NFs and GFAP contain methionine and cysteine residues with which metals are known to react (128,129). It is possible that this binding unmasks new epitopes on the autoantigen, rendering it immunogenic or more immunogenic. This provides evidence that a known neurotoxic chemical can alter the immunogenicity of antigen and enhance the magnitude of the autoimmune response. This is significant in light of the suspected involvement of heavy metals in autoimmune neurodegenerative diseases where environmental factors may play a role (see "Neurotoxic Exposures and Neuroimmune Interactions").

Autoimmune Responses and the Progression of Neurotoxicity

Numerous studies have indicated that autoantibodies may not simply represent an epiphenomenon indicative of tissue damage. Anti-MBP antibodies from MS patients have been shown to be cytotoxic and are believed to play a key role in the pathogenesis of MS (65,81). Antibodies to dihydropyridine calcium channel (the L channel) from sera of ALS and Lambert-Eaton syndrome patients are pathogenic and interfere with nerve conduction, synaptogenesis, and neurite growth (83,84). Furthermore, recent evidence indicates that autoantibodies do not interact with surface antigens but may penetrate normal cells, including neurons, to produce degeneration and apoptosis (130–132).

We have shown that the immunoglobulin fragment from lead-exposed rats attenuates presynaptic neurotransmission in isolated neuromuscular preparations (Figure 7) (133). It did not, however, block muscle responses postsynaptically, as evidenced by normal

responses to exogenously added acetylcholine. Autoantibodies to the presynaptic protein synaptotagmin were detected in these sera. Sera of unexposed rats did not block neurotransmission nor did they contain anti-synaptotagmin antibodies. Synaptotagmin is a calcium-dependent vesicle protein, a component of the docking-fusion pore machinery (134,135). Autoantibodies to this protein or other presynaptic proteins processed following axonal degeneration in sera of lead-exposed rats may interfere with neurotransmitter release. These results are not surprising with metals. Synapses are a major site of mercury neurotoxicity (136–138). Presynaptically, mercury blocks sodium and calcium channels (139). Lead blocks different types of channels (140,141) and interferes with calcium regulatory proteins such as protein kinase C and calmodulin (142,143). In the course of interactions with these target proteins and the ensuing neurodegeneration is an autoimmune response with the potential to subsequently target these neuronal mechanisms possible? This would be consistent with our studies and studies of sera of patients with Lambert-Eaton syndrome where autoantibodies to synaptotagmin, synaxin, the N, P, and Q calcium channels have been detected and shown to interfere with calcium currents and neurotransmitter release (144–151). In contrast, sera from patients with Miller-Fisher syndrome block neuromuscular transmission pre- and postsynaptically (152); sera from ALS patients (153) and diabetic BB/W rats (154) enhance calcium currents and neurotransmitter release.

In contrast to the studies with lead is the enhancement of muscle contractility to electrical stimulation of a nerve-muscle preparation and to exogenous acetylcholine in the presence of immunoglobulin from hens exposed to phenyl saligenin phosphate 21 days earlier (155,156). Sera from these hens contained antibodies to acetylcholinesterase (AChE), which would explain these responses and the inhibition of muscle homogenate

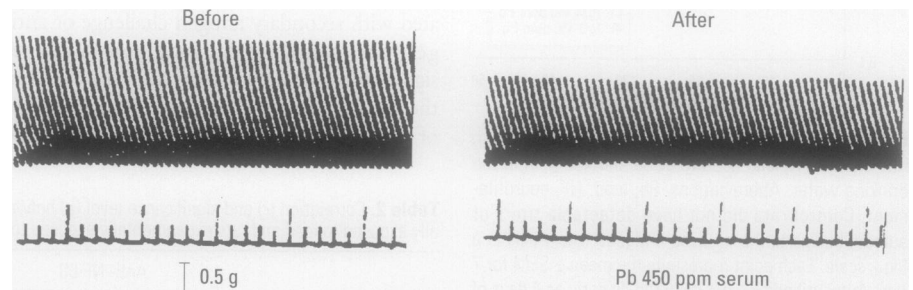


Figure 7. Sample trace of an isolated neuromuscular preparation twitch response to electrical stimulation of the nerve (acetylcholine release) in the absence (before) and presence (after) of a 150- μL IgG fragment isolated from sera of a rat exposed to 450 ppm lead for 42 days. There was an attenuation of muscle responses in the presence of the immunoglobulin fragment. Sera had detectable titers to nervous system proteins, including synaptotagmin (see text).

AChE by the immunoglobulin fragment of the sera. Pathogenic anti-AChE antibodies have been reported in several neuropathies including ALS (157–160). It should be noted that inhibition of endothelial AChE by anti-esterases increases BBB permeability (161). Sera from these same hens had detectable autoantibodies to NFs and MBP as early as seven days following exposure. Titers of NF autoantibodies significantly correlated with clinical deficits. This is consistent with the axonopathy known to be associated with some organophosphates.

Immunoglobulins to postsynaptic receptors have not yet been identified following neurotoxic exposures but are likely to play a role in neuropathy. This is recognized in myasthenia gravis with autoantibodies to the nicotinic acetylcholine (ACh) receptor (49), anti-muscarinic (m)AChR in Chagas disease (162), and anti- β -adrenoceptors in dilated cardiomyopathy (163,164).

Another pathogenic role for autoantibodies is suggested in experiments by Claudio et al. (165,166). Mice with a duplication of *d*-aminolevulinic dehydrogenase (ALAD) when exposed to lead manifested an increase in BBB permeability. These animals had significant autoantibodies to neural proteins, particularly GFAP. Immunohistochemistry of the BBB showed significant presence of IgG at the BBB. This was not observed in control mice or in mice with a single copy of the ALAD gene. This study suggests that increased BBB permeability not only exposes neural epitopes to the immune system, but that immunoglobulins gain access to the CNS. The question of whether these autoimmune responses reflect compromised immunoprivilege is partially answered by the detection of antisperm immunoglobulins in the sera of the same individuals exposed to lead and mercury (167). The blood–testes barrier is believed to serve a function similar to that of the BBB and may be affected by metals.

In addition to the above-described studies, autoantibodies to nervous system proteins have been detected in animals exposed to other inorganic and organic metals or solvents (168–71) and in humans exposed to metals or pesticides (172–174).

Conclusions and Recommendations

The studies discussed in this review illustrate the utility of the immune system's functional status for developing markers of neurotoxic effects of chemicals. They also suggest a role of environmental chemicals in the development of nervous system autoimmune disease and/or the progression of neuropathy. In the case of the autoantibody assays, these studies suggest a promising association between the appearance of autoantibody titers against

nervous system proteins and exposure to subclinical levels of known neurotoxins. Even if these humoral responses prove to be an epiphenomenon, secondary to nervous system injury, they would be useful indices of this injury. Currently, readily accessible and sensitive markers of neurotoxic insult (i.e., prior to overt changes) do not exist. This would be particularly applicable in efforts to protect children before severe irreversible damage occurs. The studies need to be expanded in terms of their validation with larger populations, delineation of the role of cellular immunity, and the correlation with neuropathology in experimental studies. Furthermore, this approach can be expanded to include other organ systems with characterized organ-specific antigens (e.g., reproductive, cardiovascular, and pulmonary systems). The use of these immunologic indicators to assess organ system integrity following chemical exposure provides a relatively simple, minimally invasive, objective means of documenting pathogenesis in human populations and animal studies. The question of autoimmune mechanisms as they relate to neurotoxicity has only begun to be addressed. Is the immune system a major effector in the progression of the symptoms associated with chemical-induced neuropathy? This question has only recently been raised, primarily from the work presented here. An unequivocal answer, however, can only be obtained by mechanistically based research addressing this question. Initially, studies of documented neurotoxic agents (e.g., metals, solvents) should be performed to address the involvement of neuroimmune interactions in the development of nervous system disease and to distinguish between autoimmunity as a phenomenon following degeneration and autoimmune disease.

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