higher on the basis of weight than the dose of tin that produced a sixfold increase of heme oxygenase activity in this study. Intestinal absorption of tin is low (*11*) but the body burden of the metal in humans is reported to equal that of cadmium and may substantially exceed that of trace metals such as cobalt, chromium, mercury, and nickel. As has been noted previously (*12*), however, the accuracy of quantitative data on inorganic tin contents of animal and human tissues, foods, and other domestic and commercial products is the subject of much doubt because of considerable loss of the metal during analytical procedures. Thus the extent of human exposure to tin is most likely greater than previously recognized.

The liver is, on a per unit basis, more active in cytochrome P-450 dependent oxidative metabolism of foreign chemicals than is the kidney (Table 1). However, the very potent inducing effect of tin on heme oxygenase in renal tissue is associated with a more exaggerated decrease in heme-dependent drug oxidation than is produced by the metal in liver. Since, as noted above, this tin-induced alteration in renal drug oxidative activity extends to other heme-dependent cellular functions as well, the potential toxicological consequences of the metal action described here merit further investigation.

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Theiler's Virus-Induced Demyelination: Prevention by Immunosuppression

Abstract. *The effect of immunosuppression with cyclophosphamide and rabbit antiserum to mouse thymocytes on demyelination induced by Theiler's virus in SJL/J mice was ascertained from Epon-embedded sections (1 micrometer) of the central nervous system. Immunosuppression not only eliminated mononuclear cell infiltrates in the spinal cord white matter, but it also prevented the occurrence of demyelination. These results suggest that demyelination in this infection is immune-mediated.*

It has been suggested that demyelination in multiple sclerosis may result from an immune-mediated response triggered by a virus infection of the central nervous system (CNS). However, evidence that a virus causes multiple sclerosis remains circumstantial (*1*). Further presumptive evidence for a viral etiology might come from the demonstration of a disease in animals like multiple sclerosis and produced by a virus. The essential criterion for such a model would be the selective pathological change of demyelination. Although there are several animal models of virus-induced demyelination that have been useful in studying the basic mechanisms of demyelination, in none has the destruction of myelin been demonstrated to be immunopathological (*2*).

Theiler's virus infection has been shown to produce primary demyelination in mice (*3*). Theiler's viruses are enteroviruses indigenous to colony-bred mice, and occasionally they may cause spontaneous paralytic disease (*4*). A study of the pathogenesis of Theiler's virus infection in 3-week-old, outbred Swiss mice has shown that limb paralysis developed 9 to 18 days after intracerebral inoculation of virus and was due to neuronal involvement (*3*). The distribution of the gray matter pathology was identical to that described for experimental poliomyelitis virus infection in monkeys (*5*). More importantly, all mice surviving CNS disease induced by Theiler's virus had persistent CNS infection, and between 1 and 6 months mononuclear cell infiltrates and demyelination were found in the spinal cord white matter (*3*). These pathologic findings have been substantiated by a temporal ultrastructural study (*6*). Although the early gray matter pathology appeared to be the result of direct cell lysis consistent with the known effects of enteroviruses in tissue culture, it seemed possible that the late developing white matter inflammation with concomitant demyelination might be immunopathological.

Preliminary immunosuppression experiments with outbred Swiss mice resulted in a uniformly high mortality before the effect of this treatment on demyelination could be evaluated (*7*). When 10- and 100-fold less virus was administered, demyelination did not regularly occur. Subsequently a number of inbred strains of mice were tested for susceptibility to Theiler's virus infection. The SJL/J strain proved a more suitable host than Swiss mice for studying the effect of immunosuppression on demyelination because (i) gray matter involvement is less severe (11 percent mortality compared to 48 percent), (ii) inflammation in the leptomeninges and white matter is more pronounced, and (iii) active demyelination occurs earlier—2 to 3 weeks after infection. By 2 months all surviving SJL/J mice develop a chronic neurologic disorder characterized by general inactivity and slowed movement, poor righting ability, and stimulus-sensitive extensor spasms of the limbs. In other respects, the pathogenesis of the infection in SJL/J mice is similar to that already described in Swiss mice.

In our experiments 3-week-old, male SJL/J mice (Jackson Laboratory) were inoculated intracerebrally with 1000 suckling mouse mean lethal doses ($SMLD_{50}$) of the DA strain of Theiler's virus. Infected mice received cyclophosphamide, rabbit antiserum to mouse thymocytes, or no treatment. Cyclophosphamide (Cytoxan, Mead Johnson) was dissolved in sterile saline immediately before use and injected by the intraperitoneal route as follows: 125 mg/kg on day 3, 50 to 75 mg/kg on day 8, and 50 mg/kg on days 12 and 17. This schedule resulted in less than 10 percent mortality in controls inoculated intracerebrally with 1 percent homogenate of normal suckling mouse brain instead of virus. That cyclophosphamide was indeed immunosuppressive in these experiments was indicated by its effect on serum antibody titers. Neutralizing antibody to Theiler's virus was determined by standard plaque reduction technique with antibody considered present when there was a 50 percent reduction in plaque-forming units (PFU). There was a threefold reduction in the mean antibody

titer of infected mice treated with cyclophosphamide (mean 1/6, range 0 to 1/8 for nine mice) compared with untreated animals (mean 1/20, range 1/4 to 1/32 for 11 mice). Undiluted rabbit antiserum to mouse thymocytes (Microbiological Associates, Bethesda, Md.) was administered intraperitoneally in 0.2-ml amounts on alternate days beginning 4 days before infection. This serum was of certified potency, and it protected 1-week-old mice from fatal lymphocytic choriomeningitis infection (Armstrong E-350 strain inoculated intracerebrally). Normal rabbit serum had no appreciable effect on the course or mortality of Theiler's virus infection.

Since there was no difference in the extent of demyelinating lesions on days 17 to 22, mice were anesthetized and either perfused with 3 percent glutaraldehyde in phosphate buffer (*p*H 7.4) or with 10 percent buffered formalin on these days. Moribund animals killed by perfusion on these days were recorded as mortalities since usually such animals in extremis died within 12 hours. Spinal cord tissue from animals perfused with glutaraldehyde was postfixed in 1 percent osmic acid and embedded in Epon, and 1-μm sections were stained with 1 percent toluidine blue. At least ten segments of each spinal cord were embedded in Epon, and a minimum of five sections from each segment were examined for evidence of demyelination. A portion of the spinal cord from each of these animals was embedded in paraffin, and 6-μm sections were stained with hematoxylin and eosin. Some animals were perfused with formalin and their tissues were only embedded in paraffin. Since staining replicate sections of paraffin-embedded tissue with specific stains for myelin and axis cylinders is a much less sensitive way to detect demyelination, only Epon-embedded material was used for this purpose.

All of the infected mice that were not given immunosuppressive treatment showed prominent collections of mononuclear cells in the leptomeninges, in perivascular sites, and infiltrating the adjacent spinal cord white matter. The majority of these inflammatory cells were lymphocytes or monocytes, although plasma cells also could be identified. In almost every 1-μm section from the spinal cords of infected mice, individual naked axons were seen scattered amid normally myelinated fibers, or clusters of naked axons were found (Fig. 1a). Naked axons were primarily concentrated in perivascular and subpial locations in association with mononuclear cells. In addition, examples of myelin stripping by invading mononuclear cells were clearly seen by light microscopy, and numerous macrophages laden with myelin debris could be found in the vicinity of demyelinating lesions. Gray matter involvement was minimal, consisted of neuronal necrosis and microglial proliferation, and was only seen in the paraffin-embedded material.

Table 1. Immunosuppression of Theiler's virus infection in SJL/J mice. Mice were inoculated with 1000 $SMLD_{50}$ of the DA strain of Theiler's virus and received cyclophosphamide, rabbit antiserum to mouse thymocytes, or no treatment until killed for histology on days 17 to 22 after infection. Since there was no significant variation in mortality between experiments, the results of two separate experiments have been combined. The effect of cyclophosphamide and the antiserum to mouse thymocytes on uninfected animals is described in the text.

Treatment	Mortality		Parenchymal inflammation		Demyelination	
	Ratio	Percent	Ratio	Percent	Ratio	Percent
None	6/39	15	9/9	100	5/5	100
Cyclophosphamide	26/34	77	1/14	7	0/8	0
Antiserum to mouse thymocytes	23/26	88	0/9	0	0/6	0

Conversely, immunosuppression resulted in a dramatic reduction in leptomeningitis and perivascular cuffs of mononuclear cells in 22 of 23 animals infected with Theiler's virus (Table 1). In most of these mice some mononuclear cells were present in the leptomeninges and in perivascular sites; however, virtually no inflammatory cells were seen invading the white matter (Fig. 1b). There was no evidence of demyelination in any of the 14 immunosuppressed mice in which sections of Epon-embedded material were examined. Neither naked axons nor extracellular myelin debris could be found in the neuropil. The single cyclophosphamide treated animal which had no reduction in inflammation was perfused with formalin; therefore, it was not examined for demyelination.

Immunosuppression not only abrogated demyelination, but it also potentiated the gray matter involvement and mortality (Table 1). In addition to increased numbers of necrotic neurons and more extensive microglial proliferation in the gray matter of immunosuppressed mice, focal areas of tissue necrosis were

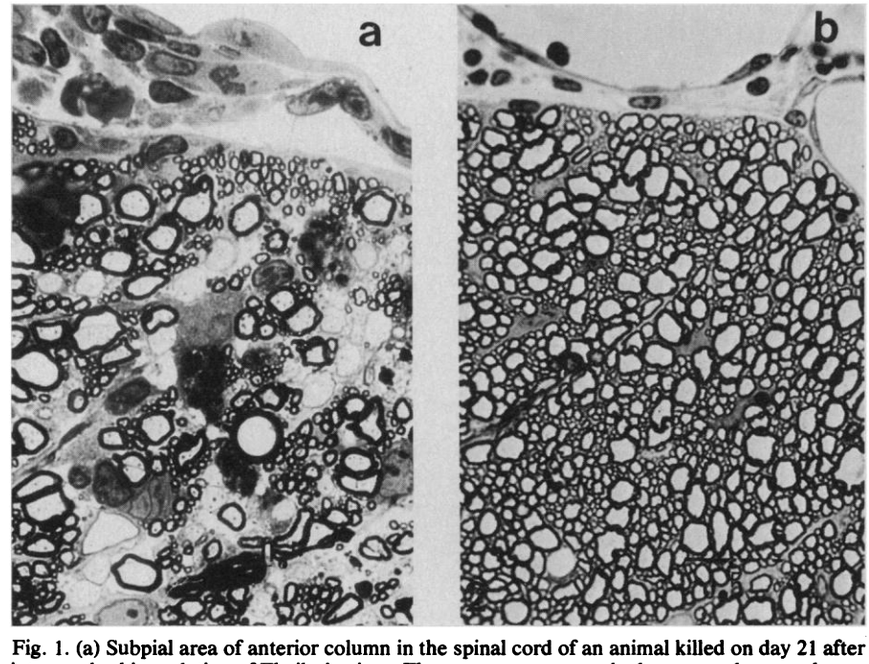

Fig. 1. (a) Subpial area of anterior column in the spinal cord of an animal killed on day 21 after intracerebral inoculation of Theiler's virus. There are numerous naked axons and macrophages laden with myelin debris, and the leptomeninges contain many mononuclear inflammatory cells (×480). (b) A comparable area from the spinal cord of an infected animal treated with cyclophosphamide and killed on day 21. There are a few inflammatory cells in the leptomeninges; however, there is no inflammation in the white matter nor myelin degeneration (×450).

also seen in the diencephalon of some mice.

Our results demonstrate that there are different mechanisms of cellular injury in the CNS in Theiler's virus infection. Both modes of immunosuppression potentiated the gray matter involvement, which indicates that the virus produces a lytic infection of neurons. This action of Theiler's virus is consistent with the known effects of enteroviruses in cell culture (*8*). In contrast, immunosuppression prevented demyelination which suggests that this white matter lesion is immune mediated. It is not possible to determine the role of humoral or cell-mediated immunity in the pathogenesis of demyelination from the use of cyclophosphamide since it has a depressive effect on both (*9*). However, administration to infected mice of rabbit antiserum to mouse thymocytes also prevented demyelination, thus favoring T cell involvement.

An immunopathology is consistent with certain other features of this infection. The occurrence of spasticity 2 months after infection when there is maximal pathologic involvement suggests that the inflammation seen in the CNS functions not only in host defense, but may in fact play a role in disease production. Moreover, the ultrastructure of the demyelinating lesions is also suggestive of immunopathologic disease because of the similarity to experimental allergic encephalomyelitis (EAE) (*10*). In both EAE and this infection there is a constant association of demyelinating lesions with mononuclear cell infiltrates, identical patterns of myelin damage consisting of vesicular disruption of myelin and stripping of myelin by invading mononuclear cells, and the absence of morphologic evidence of direct oligodendrocyte injury (*6*).

Although immune-mediated disease produced by a nonbudding virus might be considered at variance with current dogma, there is a precedent for enterovirus-induced immunopathology. Woodruff and Woodruff have shown that T cells are necessary for tissue injury to occur in the hearts of mice infected with Coxsackie B3 virus (*11*). Although the Coxsackie virus cardiomyopathy is chronic, virus replication in the heart has not been demonstrated after the first week, which is in contrast to the persistent infection in the CNS in mice infected with Theiler's virus. There are a number of proposed and documented immunologic mechanisms of virus-induced cell injury (*12*); however, the exact way in which the host immune response produces demyelination in Theiler's virus infection remains to be elucidated. Because of the failure to detect more than an occasional cell containing virus antigen ("viral targets") in the white matter by fluorescent antibody staining in this infection, it is intriguing to speculate that myelin damage may be a nonspecific consequence of the interaction between antibodies or sensitized mononuclear cells and virus. In this circumstance virus may merely be present in the vicinity of myelinated axons which are then damaged by the immune response directed to virus antigen ("bystander effect"). Recently, Wisniewski and Bloom have proposed such a mechanism to explain the occurrence of central and peripheral nervous system demyelination following local injection of mycobacterial protein into the nervous systems of guinea pigs sensitized to this antigen (*13*).

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Insulin-Dependent Diabetes: A Disease of Autoaggression

Abstract. *Lymphocytes from patients with insulin-dependent diabetes demonstrated significant cytoadherence and cytotoxicity against human insulinoma cells in vitro as compared to lymphocytes from normals. Complement was not involved in insulinoma cell destruction. The findings suggest that insulin-dependent diabetes may be a disease of autoaggression.*

Circumstantial evidence suggests that the pathogenesis of insulin-dependent diabetes (IDD) may be autoimmune in nature in some instances. In the early stage of disease, the pancreatic islets of Langerhans are often infiltrated with lymphocytes (*1, 2*). As the disease progresses, the insulin-producing cells (β cells) disappear, while the islets become small and atrophic (*1, 3*). The clinical association between IDD and autoimmune diseases such as pernicious anemia, antibody-positive thyroid disorders, myasthenia gravis, idiopathic hypoparathyroidism, and Schmidt's syndrome, as well as high frequencies of organ-specific antibodies, has been reported among IDD patients (*4*). Evidences of delayed type hypersensitivity reactions to a variety of insulins, and homologous and heterologous pancreatic extracts, have also been found (*5*), but direct evidence of autoimmunity in IDD has been lacking. Recently, several groups were able to demonstrate a variable frequency (10 to 50 percent) of circulating antibody against human β cells in serums of IDD patients (*6*). Utilizing islet cell adenoma cells as antigen, it has been possible to demonstrate immunoglobulin M and immunoglobulin G antibodies in the serums of 34 of 39 IDD patients (*7*). However, the mechanisms of β cell destruction have not been delineated. It is noteworthy that HL-A8 and W-15 antigens have been clearly associated with IDD, while HL-A8 antigens have also been linked with Grave's disease and idiopathic Addison's disease. The latter disorders have been considered to have an autoimmune pathogenesis (*8*).

Using human insulinoma cells as model β cells (*7*), we have developed an in vitro assay of lymphocyte-mediated cytotoxicity. Insulinoma cells were adapted to culture with L.Y. media with 20 percent human serums (*9*) and maintained for more than 30 passages. The