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

cells appeared to be homogenous and regularly produced insulin at concentrations of 10 to 20 microunits per milliliter of culture medium per 6.0×10^6 cells after 3 days of subculture. Lymphocytes from 23 patients (ages 2 to 20 years) with clinically established IDD of 0 to 16 years duration (one never received insulin before this study) and from 12 normal individuals (ages 3 to 28) were separated from heparinized whole blood by using Ficoll-Hypaque density gradient (*10*). Sixteen of 21 IDD patients tested had significant levels of circulating antibody against insulinoma cells (*7*). A microcytotoxicity assay (*11*) modified to utilize tissue culture tubes and counting on a hemocytometer was found to be useful particularly for pediatric patients. The insulinoma cells were tested with each subject's samples by using the following conditions: (i) lymphocytes alone, (ii) lymphocytes plus serum, and (iii) serum plus complement. Complement in each test serum was inactivated by heating at 56°C for 30 minutes. Fresh guinea pig serum was used as a source of complement. The ratio of lymphocytes to insulinoma cells was maintained at 50 : 1. The serums were added at final dilutions of 1 : 5. These conditions permitted the observation of three possible mechanisms of immunological cell injury: (i) by sensitized lymphocytes, a presumed thymus-derived (T) lymphocyte function; (ii) by antibody-dependent lymphocytes which involve Fc receptor–carrying lymphocytes; and (iii) by complement-fixing antibody.

All the cultures were maintained in 10 by 75 mm sterile culture tubes (Falcon Plastics) at 37°C in a 5 percent CO_2 incubator. The cells were removed at 18, 40, and 64 hours and observed in counting chambers for numbers of cytoadherent and dead insulinoma cells. Insulinoma cells with adhesions of one or more lymphocytes were considered positive for cytoadherence (Fig. 1, A and B). Cell death was estimated by eosin exclusion. At least 200 insulinoma cells were counted and percentages of cytoadherence and cytotoxicity were determined. A control culture of insulinoma cells alone consistently showed less than 1 percent of cell death.

As shown in Fig. 2 the mean percentage of cytoadherence of lymphocytes from IDD patients to insulinoma cells was four- to fivefold greater than that from normal individuals. This difference was observed as early as 15 hours of incubation and persisted up to 64 hours. Of 23 IDD cases, 18 showed more than 10 percent cytoadherence as compared to none of the 12 controls. The rosette formations of multiple lymphocytes attached to insulinoma cells (Fig. 1B) were consistently seen only with cells from IDD patients.

The cytotoxicity of lymphocytes from IDD on the insulinoma cells was also significantly greater than that of normal lymphocytes (Fig. 3). With normal lymphocytes the frequency of dead model β cells was less than 9 percent at 40 hours, whereas with the IDD lymphocytes, the frequency was 24 percent. These results suggest that cell to cell interaction between lymphocytes and insulinoma cells was important in subsequent cell damage of insulinoma cells, although the possibility that cytotoxic factors were released from sensitized lymphocytes cannot be excluded.

In observing patients individually, we

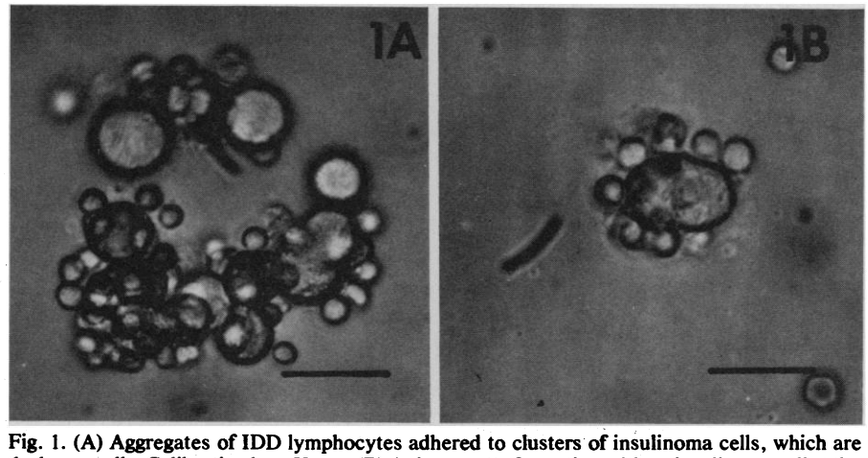

Fig. 1. (A) Aggregates of IDD lymphocytes adhered to clusters of insulinoma cells, which are the larger cells. Calibration bar, 50 μm. (B) A ring rosette formation with an insulinoma cell at the center surrounded by IDD lymphocytes. Calibration bar, 50 μm.

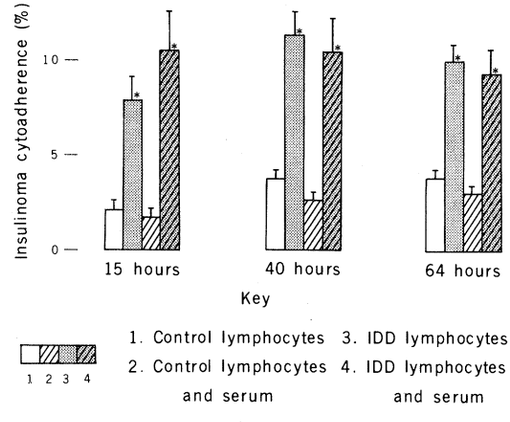

Fig. 2. Results of human insulinoma cytoadherence test observed at different incubation periods. The study involved lymphocytes from 23 IDD patients and 12 normal controls. The bars represent the means, and the vertical lines, standard errors of the means. The differences between diabetic (*) and corresponding control lymphocytes are significant ($P < .01$).

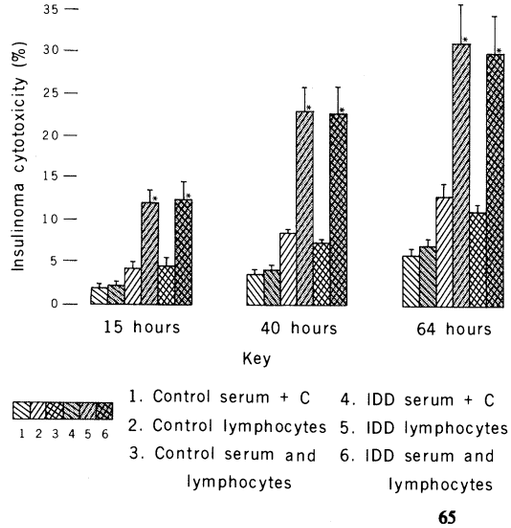

Fig. 3. Results of human insulinoma cytotoxicity test observed at different incubation periods. The various testing conditions were to define (i) complement-activated, (ii) T cell, and (iii) B or K cell cytotoxicity. The study included lymphocytes from 23 IDD patients and 12 normal controls. The bars represent the means, and the vertical lines, standard errors of the means. The differences between diabetic (*) and corresponding control lymphocytes are significant ($P < .01$). Abbreviation: C, complement.

found that 15 of 23 IDD lymphocyte preparations showed at least 21 percent insulinoma cell death as compared to none in 12 normal controls. Among positive cases, 2 showed predominantly lymphocyte-mediated cytolysis, 2 showed antibody-dependent lymphocyte cytolysis, and 11 demonstrated that both cytotoxic mechanisms were operating. In our test system, serum plus complement had no effect on cytolysis (Fig. 3).

In order to study the cytotoxic activity of subpopulations of lymphocytes, cells from seven patients with positive reactions were further fractionated with sheep erythrocytes and Ficoll-Hypaque gradient into rosette-forming cells and non-rosette-forming cells. Those lymphocytes which have receptors to form sheep erythrocyte rosettes in 2 hours at 4°C were operationally defined as T lymphocytes, while non-rosette-forming cells were defined as bone marrow–derived lymphocytes (B cells) or cells of third population with Fc receptors (K cells) (*12*). The assays were performed with insulinoma cells cultured with rosette-forming cells alone or non-rosette-forming cells plus serums. The two patients who showed predominant lymphocyte-independent cytolysis had a significant enhancement of cytotoxicity by T cell enrichment. Another patient, who showed antibody-dependent lymphocytolysis, had enhanced cytolysis with non-rosette-forming cells plus serums. The other four cases showed cytotoxicity of equal degree by two subpopulations, indicating both antibody-independent (T cells) and antibody-dependent (B or K cells) lymphocyte-cytotoxic processes.

Our study indicates that lymphocytes, and not antibodies, in IDD patients are the primary aggressors in the process of pancreatic β cell autoaggression. Thus, cell-mediated immunity against β cells may be an important pathogenic mechanism in IDD. These findings would explain the pancreatic lesions of IDD that are characterized by marked infiltration of mononuclear cells, which are commonly seen in other autoimmune endocrine diseases. The fact that one patient already showed T cell cytotoxicity even before insulin therapy may imply that the sensitization to insulin is not a primary factor. One intriguing question which remains to be answered is whether infectious agents, particularly viruses (*13*), might trigger this process of autoimmune β cell destruction in a genetically predisposed individual. This possibility needs further exploration since, in the future, preventive measures might be developed for IDD.

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Palaeosclerotium, a Pennsylvanian Age Fungus Combining Features of Modern Ascomycetes and Basidiomycetes

Abstract. *Fruiting bodies described previously as sclerotia of* Palaeosclerotium pusillum *are cleistothecium-like, enclosed structures, containing spores within an ascus. The cleistothecia are composed of hyphae having dolipore-like septa and are attached to vegetative hyphae having clamp connections. The unique combination of ascomycete-like reproductive bodies and basidiomycete-like hyphae present in this Pennsylvanian fungus suggests that there already existed, during the Middle Pennsylvanian period, a group of fungi intermediate between the Ascomycetes and Basidiomycetes.*

Permineralized fossil fruiting bodies found associated with Pennsylvanian plants (*1*) combine features of the traditional classes of higher fungi, Ascomycetes and Basidiomycetes. The cleistothecial fruiting body (Fig. 1A) and sac-like ascus (Fig. 1A, arrow; enlarged in Fig. 1B) are characteristic features of sexual reproduction in the class Ascomycetes. In contrast, the clamp connections (Fig. 1C) and complex septal pores (Fig. 1F) identify the hyphae with the class Basidiomycetes.

The fruiting body illustrated is a small, spherical, enclosed (cleistothecium-like) structure (624 μm in diameter) composed of a compact outer peridium (112 μm thick) of pseudoparenchymatous tissue and a central zone (400 μm in diameter) of polyhedral tissue (Fig. 1A). The peridium of pseudoparenchymatous cells is continuous to the exterior with vegetative hyphae (Fig. 1, A and C). The central polyhedral tissue (Fig. 1A) is composed of ramified hyphae that average 3.5 μm in diameter (Fig. 1E). Therefore, the fossil form has a dense, compact, two-zoned spherical fruiting body situated within a mycelium. The fossil resembles structures found within the ascomycete family Trichocomataceae (*2*).

The vegetative mycelium is most remarkable because of the presence of clamp connections. A number of such clamp connections are present at the cross walls of the hyphae, which organically attach to the pseudoparenchymatous cells of the outer zone of the fruiting body (Fig. 1C). One well-preserved clamp connection is seen to have features commonly recognized in clamp connections of extant basidiomycete fungi (Fig. 1C, arrow). Two septa are present; one is positioned within the base of the clamp and the second partitions the hypha just below the opening circumscribed by the clamp.

The central, polyhedral tissue of the fruiting body contains numerous sporangia (Fig. 1, A, B, and D). These glo-