VIRUS-INDUCED DIABETES MELLITUS

XX. Polyendocrinopathy and Autoimmunity

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Insulitis has been observed in the pancreas of children with insulin-dependent diabetes mellitus (IDDM)[1] of <6-mo duration (1). The cause of the insulitis is not known, but it has been suggested that viral infections and/or immunopathologic mechanisms may play a role in the etiology and pathogenesis of IDDM (2). Recently, autoantibodies that bind to cytoplasmic and surface antigens on pancreatic islet cells have been found in the sera of many patients with IDDM, but not in the sera of non-insulin-dependent diabetics (3–6). Some patients with IDDM also suffer from a more generalized polyendocrine disorder and have autoantibodies in their sera that react with other hormone-producing cells (e.g., thyroid, gastric mucosa, adrenal, pituitary) (3, 4, 7, 8).

What it is that triggers the production of autoantibodies in IDDM is not known, but viruses have often been suspected as a cause of autoimmune disease (9). Autoantibodies to host antigens (e.g., DNA, lymphocytes, erythrocytes, immunoglobulins, smooth muscle, myelin) have been observed after certain viral infections (10–14), and Epstein-Barr virus is known to be a polyclonal activator (15). Transient antibody to cytoplasmic islet cell antigens also has been observed in some patients after mumps infection (16).

This investigation was initiated to see whether viruses that produce diabetes in animals also would trigger an autoimmune response. Earlier studies from our laboratory showed that reovirus type 3 could infect pancreatic beta cells in mice and produce diabetes (17). We now report that reovirus type 1 also produces diabetes in mice, but in addition triggers a polyendocrine disease and induces autoantibodies to insulin and growth hormone (GH).

Materials and Methods

Animals. NIH-Swiss and SJL/J mice were obtained from the National Institutes of Health (NIH) Small Animal Section, Bethesda, Md. and The Jackson Laboratory, Bar Harbor, Maine, respectively. Newborn mice (3–6 d old) of both sexes were inoculated intraperitoneally or intracerebrally with 5×10^5 or 5×10^4 plaque-forming units (pfu) of virus, respectively. Except where indicated, NIH-Swiss mice, inoculated intraperitoneally, were used.

Viruses and Cell Cultures. Reovirus type 1 (Lang strain) and reovirus type 3 (Dearing strain)

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[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; EMC, encephalomyocarditis virus; FITC, fluorescein isothiocyanate; GH, growth hormone; HRPO, horseradish peroxidase; IDDM, insulin-dependent diabetes mellitus; NIH, National Institutes of Health; pfu, plaque-forming units; RIA, radioimmunoassay.

were passaged in SJL mouse pancreatic beta cell cultures (18) unless indicated otherwise. Recombinant viruses 1.HA3, 3.HA1, and clone 94 were kindly provided by Dr. Bernard Fields, Harvard Medical School, Boston, Mass. and passaged in L-929 cells (19, 20). All titrations were performed on L-929 monolayers using an agar overlay. The D clone of encephalomyocarditis (EMC) virus (21) was grown and titrated in secondary mouse embryo cells.

Anti-Viral Sera. Type-specific goose sera against reovirus type 1 and reovirus type 3 were obtained from the Research Resource Branch, National Institute of Allergy and Infectious Diseases, NIH and used to identify the different reoviruses by means of a standard hemagglutination inhibition test with human type O erythrocytes for reovirus type 1 and bovine erythrocytes for reovirus type 3 (20). Rabbit antibody to reovirus type 1 or mouse antibody to reovirus type 3 (Microbiological Associates, Walkersville, Md.) was used in direct immunofluorescence tests after labeling with fluorescein-isothiocyanate (FITC) by standard methods (17).

Detection of Viral Antigens by Direct Immunofluorescence. Cryostat sections (5 μm thick) of tissue from infected and uninfected animals were fixed in acetone for 5 min at room temperature, flooded with FITC-labeled anti-viral serum, incubated at 7°C overnight, and examined by fluorescent microscopy (17).

Detection of Autoantibodies by Indirect Immunofluorescent. Unfixed frozen sections of pancreas, pituitary, stomach, thyroid, adrenals, ovaries, and testes were obtained from uninfected adult mice and incubated for 45 min at 37°C with various dilutions of sera from infected or uninfected mice. After gentle washing, the sections were further incubated overnight at 7°C with an appropriate dilution of FITC-labeled rabbit anti-mouse IgG (heavy and light chain; Miles Laboratories Inc., Elkhart, Ind.). Background staining was assessed by using normal mouse sera.

In other experiments, organs from uninfected mice were fixed in Bouin's solution for 6 h (room temperature), washed five times in 70% ethanol, embedded in paraffin, and sectioned. The sections were then incubated with serum from infected or uninfected mice and stained with FITC-labeled anti-mouse IgG as described above. Autoantibodies could be detected by both methods but, in general, Bouin's-fixed tissue yielded better results than unfixed frozen sections.

Antibodies to Hormones as Measured by Enzyme-linked Immunosorbent Assay (ELISA). The antigens used in these tests were highly purified preparations of rat GH (GH-RP-1 or iodination-grade GH-I-4 obtained through the courtesy of Dr. A. F. Parlow, National Pituitary Agency, Baltimore, Md.) and rat insulin (Novo Industries, Copenhagen, Denmark). Microelisa plates (Cooke Engineering Co., Alexandria, Va.) containing 96 wells were coated with diluted hormones (i.e., 9.2 μg/well of GH or 0.8 μg/well of insulin) in 0.1 ml of carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C. After three washes, serial twofold dilutions of sera from infected or uninfected mice in phosphate-buffered saline-Tween 20 were added to the wells and incubated for 2 d at 4°C. Pilot experiments showed that this long incubation was required for optimal sensitivity. The wells then were washed three times and the amount of antibody bound to the antigen was measured by adding an excess of horseradish peroxidase (HRPO)-conjugated anti-mouse immunoglobulin (45 min at room temperature) and orthophenylenediamine (Aldrich Chemical Co., Milwaukee, Wisc.) as substrate (22). The class of anti-hormone antibody was assessed using HRPO-conjugated anti-mouse IgG (Miles Laboratories Inc.) or anti-mouse IgM (Litton Bionetics, Kensington, Md.) conjugated in our laboratory with HRPO (electron microscope grade; Tousimis Co., Rockville, Md.) by the periodate method (23). Specificity of the assay was checked with mouse sera (from infected and uninfected animals) using a battery of protein antigens (e.g., prolactin, glucagon, albumin, ribonuclease B) and comparing the results with specific antisera to GH and insulin. Absorbance was read with a microplate reader (Flow Laboratories, Inc., Rockville, Md.) and antibody titer to hormones was expressed as the reciprocal of the highest dilution of serum yielding a sample:control ratio of 2.0 or greater. By this method, hyperimmune sera to GH and insulin gave titers of 800 and 32,000, respectively (A. Toniolo, manuscript in preparation).

Measurement of Hormones by Radioimmunoassay (RIA). Insulin and glucagon were extracted from mouse pancreas (18, 24) with slight modifications including the use of a protease inhibitor (aprotinin; Sigma Chemical Co., St. Louis, Mo.). Immunoreactive insulin was measured by RIA using mouse insulin as a standard (18). Glucagon determinations were kindly performed

by Dr. Lillian Recant, Veterans Administration Hospital, Washington, D. C. GH was measured by a double-antibody RIA (25) using ^{125}I-labeled rat GH. Individual mouse sera were tested after ultricentrifugation (130,000 *g* for 90 min) to eliminate immune complexes.

Prolactin Receptor Assay. Prolactin receptors were evaluated by measuring the amount of ^{125}I-labeled ovine prolactin (National Pituitary Agency) that specifically bound to mouse liver membrane preparations (26) from infected and uninfected mice. In brief, labeled prolactin (80,000 cpm) was incubated with liver membrane (0.14–0.31 mg protein) in final vol of 0.5 ml. After overnight incubation at room temperature, the reaction was stopped by adding 1.0 ml of Tris buffer at 4°C. The tubes were centrifuged at 2,500 *g* for 20 min, the pellets washed two times, and the radioactivity measured. The amount of radiolabeled prolactin (cpm) bound in the presence of 0.5 μg unlabeled hormone was used as the value for nonspecific binding and was subtracted from total binding (26).

Glucose Assay. Glucose in the blood was measured by the glucose oxidase assay with *o*-dianisidine dihydrochloride as the indicator dye. Blood for glucose tolerance tests was obtained 60 min after i.p. injection of 2 mg of glucose/g body weight (17).

Pathology. For light microscopy, tissues were fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. For electron microscopy, tissues were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, embedded in Epon 812 (Tousimis Co.), stained with uranyl acetate and lead citrate, and examined with a Philips 201 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.).

Results

Hyperglycemia and Infection of the Islets of Langerhans. Mice were infected with reovirus type 1 and at different times thereafter, glucose, insulin, and glucagon were measured. The data in Fig. 1 A and B show that although only a small percentage of mice had elevated nonfasting glucose levels, abnormal glucose tolerance tests were observed in up to 45% of the animals with some values >400 mg/dl. Pancreatic immunoreactive insulin was depressed beginning at 3 d after infection and remained lower than in controls throughout the experiment (Fig. 1 C). In contrast, plasma glucagon was unchanged or slightly elevated (Fig. 1 D).

Evidence that the metabolic alterations were secondary to changes in the pancreatic islets of Langerhans came from histologic, immunofluorescence, and electron microscopic studies. By light microscopy, focal degenerative cytoplasmic changes and pynotic nuclei were found in about one-fourth of the islets between 5 and 7 d after infection (Fig. 2 A). 7–9 d after infection, focal necrosis and disruption of islet architecture were observed in approximately one-fifth of the islets. Mononuclear and occasional polymorphonuclear cells also were observed infiltrating islets and the interstitial connective tissue; later (i.e., 3 wk), large numbers of plasma cells surrounded and infiltrated the peripheral area of many islets (Fig. 2 B). In the exocrine portion of the pancreas, minimal focal necrosis of acinar tissue and minimal periductal cell infiltrates with some degree of ductal obstruction were observed in many of the mice.

By immunofluorescence, viral antigens were found in the islets of Langerhans beginning at 5 d after infection, but generally were not detected after 12 d. The intensity of fluorescence was greatest at 7 d and at that time, approximately one-half of the islets contained some viral antigens. In general, <20% of the cells within any one islet were positive by immunofluorescence but, in some cases, almost the entire islet showed bright fluorescence (Fig. 2 C). By electron microscopy, viral particles were seen primarily in beta cells, but also in some alpha and delta cells (Fig. 2 D and E).

Retarded Growth and Infection of the Anterior Pituitary. During the early phase of the

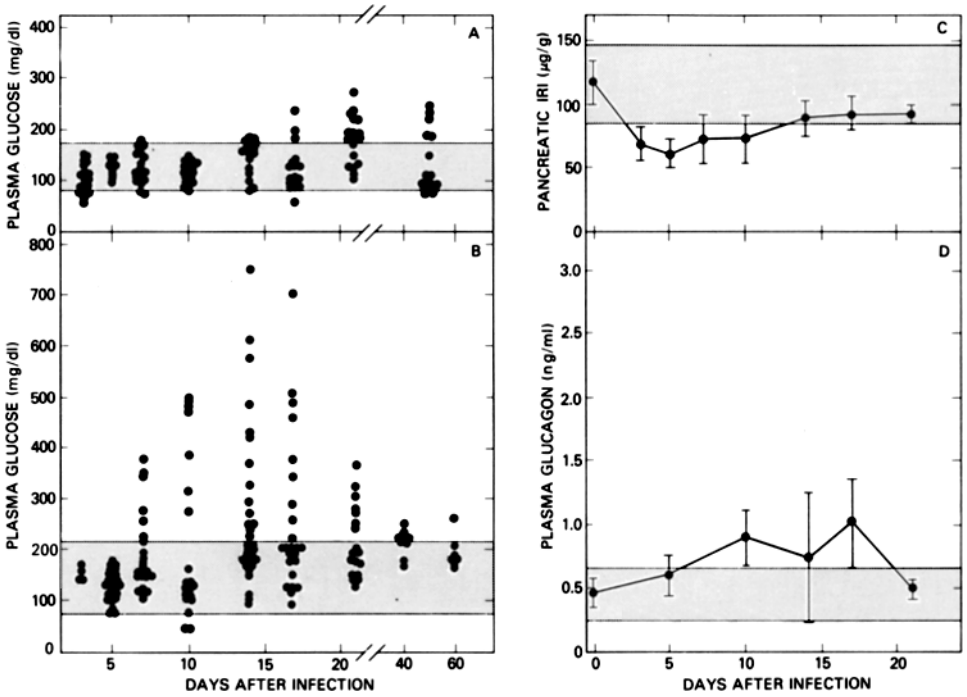

FIG. 1. Alterations of glucose, insulin, and glucagon after infection with reovirus type 1. Shaded areas represent the mean of uninfected mice ± 2 SD. In panels A and B, each point represents the glucose concentration of an individual mouse. In panels C and D each point represents the mean ± SD of seven to ten infected mice. (A) Nonfasting glucose, (B) glucose tolerance test, (C) pancreatic immunoreactive insulin (IRI), and (D) plasma glucagon.

work, we observed that a number of mice were runted. To study this in more detail, mice were infected and at different times thereafter weights were determined (Fig. 3) and clinical evaluations made. At 14, 21, and 35 d after infection 49, 48 and 25% of reovirus-infected mice, respectively, showed significant retardation in growth (i.e., 2 SD below the mean of the uninfected mice) as compared with uninfected controls. Moreover, at 2–3 wk after infection, ~75% of the mice with retarded growth also displayed oily hair and/or steatorrhea; 45% showed thinning of hair and/or alopecia; and ≥20% had ascites.

The retardation in growth raised the possibility that the virus might be damaging cells in the pituitary. At 7–14 d after infection, light microscopy revealed inflammatory infiltrates of mononuclear cells and focal areas of coagulation necrosis in the anterior lobe of the pituitary, but not in the intermediate or posterior lobes. Immunofluorescence showed that viral antigens also were present in the anterior, but not the posterior lobe of the pituitary (Fig. 4 A), and electron microscopy revealed viral particles in growth hormone-producing cells (Fig. 4 B), especially in the areas where focal necrosis was observed.

To see whether the virus-induced pathology in the pituitary might be associated with alterations in hormones, GH levels in the blood were measured. Although considerable variation was observed among animals, a significant decrease in the mean GH concentration was found at 14 d after infection in runted as compared to

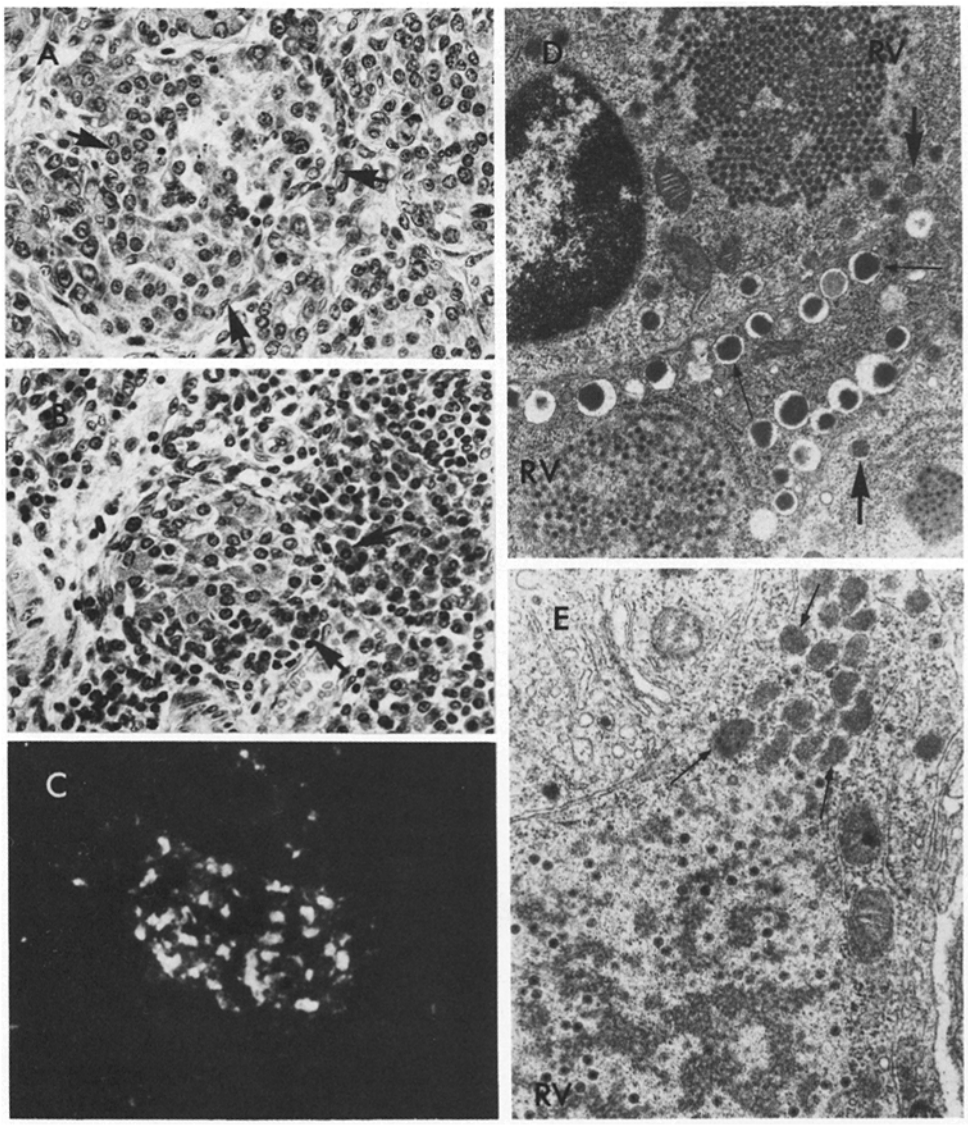

FIG. 2. Microscopic changes in pancreatic islets of Langerhans of mice infected with reovirus type 1. (A) Focal degenerative cytoplasmic changes and pynotic nuclei with loss of normal islet architecture seen 7 d after infection. Arrows mark periphery of islet; hematoxylin and eosin (HE). × 550. (B) Mononuclear cells, especially plasma cells (arrows), are seen infiltrating and surrounding an islet at 21 d after infection. HE. × 550. (C) Frozen section from pancreas taken 7 d after infection and stained with FITC-labeled antibody to reovirus type 1. Viral antigens seen in many cells throughout the islet. The surrounding acinar cells are relatively free of viral antigens. × 340. (D) Electron micrograph of cells in mouse pancreas 7 d after infection with reovirus type 1. Pancreatic beta cell with insulin-containing granules (small arrows) is located between two alpha cells containing characteristic glucagon-containing granules (large arrows). The three cells contain cytoplasmic reovirus particles (RV) in different stages of development. In the alpha cell (top), reovirus particles are in crystalline array. In the beta cell (middle), the virus particles form a reticulo-granular matrix along lines of aggregated matrix material. In the alpha cell (bottom), virus particles are seen in a phagocytic vacuole. × 14,000. (E) Pancreatic delta cell with characteristic somatostatin-containing granules (arrows) and reticulo-granular matrix containing developing reovirus particles (RV). × 20,000.

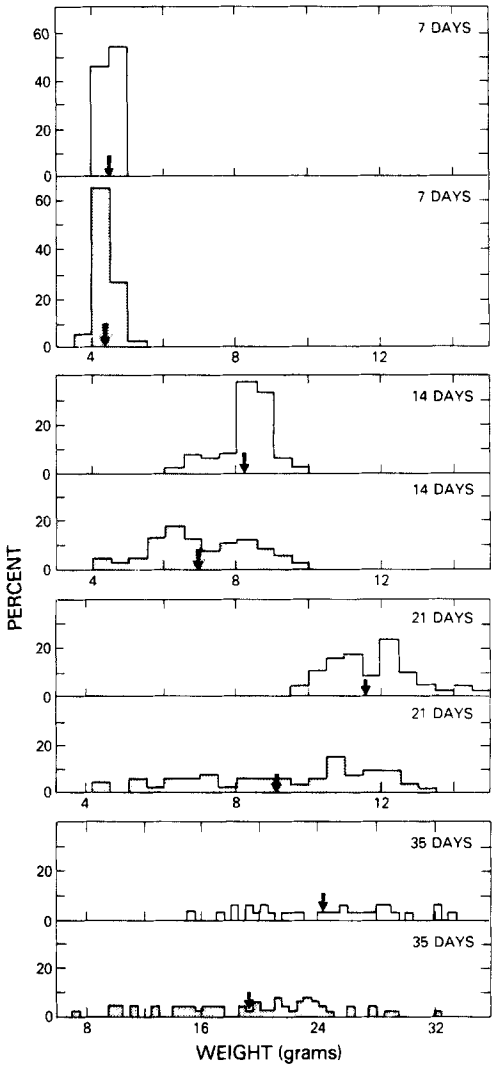

FIG. 3. Growth retardation of mice infected with reovirus type 1. Mice were weighed at the times indicated and the weight distribution plotted as a percentage. Open bars, uninfected mice; shaded bars, infected mice. Arrows represent the mean weight of each group. Data from several experiments were pooled. The number of mice in the uninfected groups on days 7, 14, 21, and 35 were 26, 53, 64, and 31, respectively. The number of mice in the infected groups on the same days were 58, 92, 70, and 51, respectively.

non-runted mice or uninfected controls ($P < 0.001$) (Fig. 5 A). Because GH concentration is one of the factors that regulates prolactin receptors (26), we also measured the binding of ^{125}I-prolactin to receptors on liver membranes. The data in Fig. 5 B show that at 14 d after infection, the binding of prolactin was reduced by almost 60% in runted as compared with nonrunted mice or uninfected controls ($P < 0.005$).

Autoantibodies. Because runting can be immunologically induced (27, 28) and autoimmunity may contribute to the pathogenesis of IDDM (6), we decided to look for autoantibodies in the sera of infected mice. Sera were tested by indirect immu-

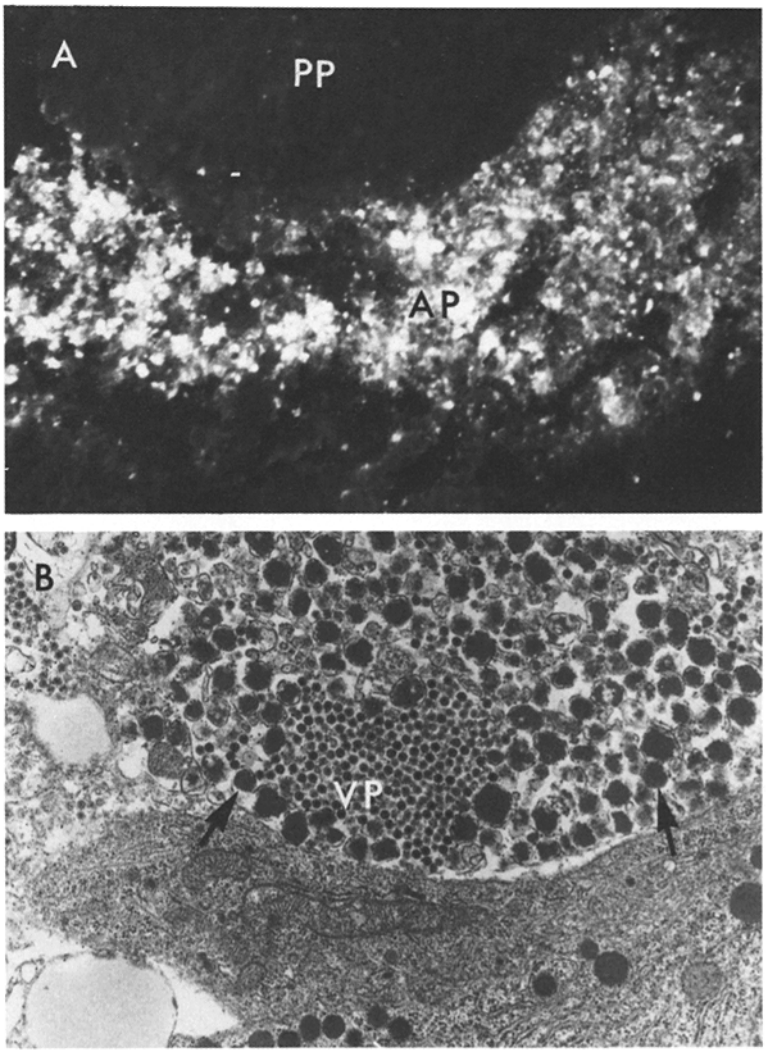

FIG. 4. Viral antigens and viral particles in the anterior pituitary of mice 7 d after intracerebral infection with reovirus type 1. (A) Frozen section stained with FITC-labeled antibody to reovirus type 1. Viral antigens are found in the anterior (AP), but not in the intermediate or posterior (PP) lobes of the pituitary. × 240. (B) Electron micrograph of anterior pituitary showing a cell with growth hormone-containing granules (arrows) and reovirus particles (VP) that are either single or in crystalline array. × 14,000.

nofluorescence for the presence of autoantibodies to pancreas, pituitary, stomach wall, intestine, adrenals, thyroid, ovaries, and testes obtained from uninfected mice. By immunofluorescence, autoantibodies were found that reacted with cells in the islets of Langerhans, anterior pituitary, and stomach wall. As seen in Fig. 6A, sera from reovirus-infected mice stained islet cells, but not the surrounding acinar tissue. Particularly brilliant fluorescence was sometimes observed at the periphery of the islets where alpha and delta cells are located. Staining also was observed in the anterior, but not in the intermediate or posterior lobes of the pituitary (Fig. 6B).

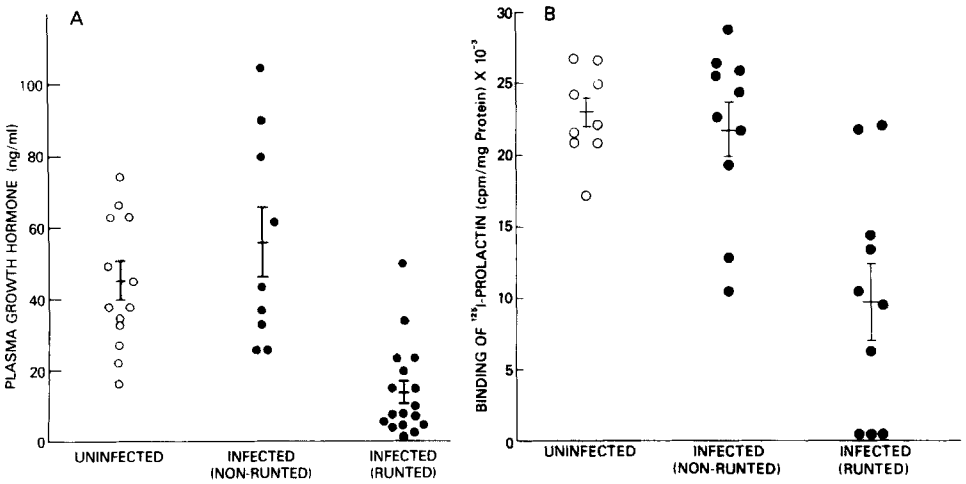

FIG. 5. Effect of reovirus type 1 on growth hormone concentration and prolactin receptors. Mice were infected intraperitoneally with reovirus, and 14 d later all the animals were weighed. Mice were scored as runted if their body weights were ≥2 SD below the mean of uninfected controls. Each point represents an individual animal. The mean ± SE is shown by vertical lines and bars. (A) Concentration of growth hormone in plasma; (B) binding of ^{125}I-prolactin to receptors on liver membranes.

Staining of the stomach wall was usually weaker and primarily localized to the crypts, possibly parietal cells (data not shown). No staining was observed with the intestines, adrenals, thyroid, ovaries, or testes.

To determine the nature of the antigens involved, sera that contained autoantibodies were absorbed with growth hormone or insulin. As seen in Table I, autoantibodies to the anterior pituitary were absorbed by rat GH, which antigenically is closely related to mouse GH, but not by bovine GH, which antigenically is poorly related to mouse GH (29). Absorption with insulin also failed to reduce the titer of this autoantibody. In contrast, sera containing autoantibodies to islet cells could be absorbed by insulin, but not by rat GH.

To be sure that the antibodies to GH and insulin were being induced by the viral infection and not by contaminating hormones in the viral inoculum, mice were infected with homogenates of uninfected beta cells or L cells that had been used to propagate the virus. None of these preparations induced autoantibodies to GH or insulin. Moreover, as seen in Table I, mice infected with the diabetogenic D variant of EMC virus (21) failed to induce autoantibodies. Attempts to induce autoantibodies in newborn mice with two other viruses (i.e., mumps and Coxsackie B4), thus far have been unsuccessful.

The time of appearance and duration of autoantibodies are shown in Table II. By immunofluorescence, antibodies to pituitary cells were found in 100% of the mice at 7 d after infection and 63% of the mice were still positive at 40 d. Antibodies to islet cells were found in only 44% of the mice when measured at 14 d, and 33% remained positive at 40 d. In general, sera from infected mice that were runted produced stronger fluorescence than sera from infected non-runted mice (data not shown).

Additional information on the nature of the antigens and the titer of the autoantibodies comes from studies in which purified GH or insulin was bound to plastic wells

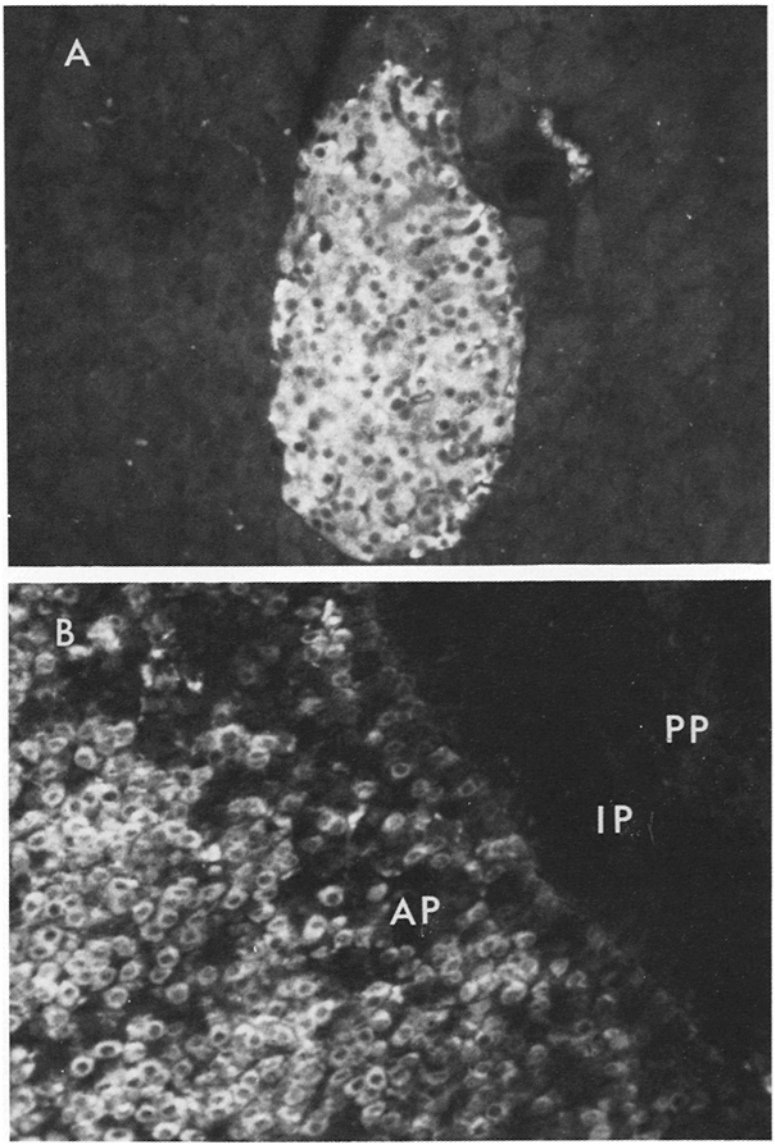

FIG. 6. Autoantibodies in sera of reovirus-infected mice detected by indirect immunofluorescence. (A) Section of normal mouse pancreas (Bouin's fixed) incubated with sera obtained from runted mice 14 d after infection. Photomicrograph shows cytoplasmic staining of cells in the islets of Langerhans with little if any staining of surrounding acinar cells. × 340. (B) Section of normal mouse pituitary (Bouin's fixed) incubated with sera obtained from runted mice 14 d after infection. Brilliant fluorescence is seen in the anterior lobe of the pituitary (AP). The intermediate (IP) and posterior (PP) lobes show little if any fluorescence. × 680.

and the antibodies measured by ELISA. As seen in Fig. 7A, within 10 d after infection, all of the mice had antibodies to GH with a titer ≥20 and in a few mice the titer was ≥160. After 10 d the antibody titer began to decline and at 30 d, ~60% of the mice had titers <10. Fig. 7B shows that the antibody titer to insulin ranged from

TABLE I

Autoantibodies in the Sera of Reovirus-infected Mice to Pancreatic Islets and Anterior Pituitary

Sera	Titer*	
	Anterior pituitary	Pancreatic islets
Uninfected mice	<2	<2
Reovirus type 1-infected‡		
Unabsorbed	256	16
Absorbed with bovine insulin§	256	4
Absorbed wiht bovine GH	256	ND¶
Absorbed with rat GH	4	16
EMC virus-infected‖	<2	<2

* Titer represents the reciprocal of the highest dilution of serum giving positive cytoplasmic fluorescence on Bouin's-fixed tissue from uninfected mice.
‡ Pooled sera obtained 21 d after infection with reovirus type 1.
§ Absorptions were carried out by incubating sera with insulin or GH (0.5 mg/ml) for 12 h at 4°C.
‖ Pooled sera obtained 21 d after infection with EMC virus.
¶ Not done.

TABLE II

Appearance and Duration of Autoantibodies as Determined by Immunofluoresence

Days after infection	Sera*			
	Pituitary‡		Islets‡	
	Uninfected	Infected	Uninfected	Infected
	% positive§			
7	0	100	ND‖	ND
14	0	78	0	44
21	0	75	0	28
28	0	81	0	33
40	0	63	0	33

* 3-d-old mice were infected with reovirus type 1. Uninfected mice served as controls. Individual sera from 5 to 14 mice were tested at each of the times indicated by indirect immunofluorescence.
‡ Sera were incubated with Bouin's-fixed sections of pituitary and pancreas from uninfected mice.
§ Sera at a 1:2 dilution giving cytoplasmic fluorescence were considered positive.
‖ Not done.

10 to 160, but at each of the times evaluated, fewer mice had antibody to insulin than to GH.

It is well documented that reovirus produces clinical disease when inoculated in newborn or very young mice, whereas older mice are far more resistant to the development of clinical disease (30). To see whether the capacity of reovirus to induce autoantibody to GH was age dependent, mice were infected at different times after

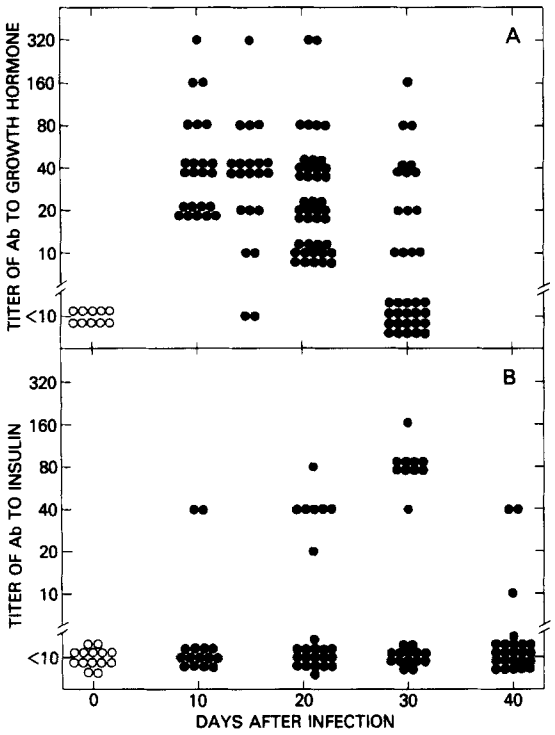

FIG. 7. Titer of autoantibodies to GH (A) and insulin (B) in the plasma of reovirus-infected and control mice as determined by ELISA. Each point represents an individual animal. (○) Uninfected mice; (●) infected mice.

TABLE III

Age-dependent Induction of Autoantibody to GH by Reovirus Type 1

Age at infection	Days after infection		
	14	21	30
d	*% positive**		
3	100	100	100
14	100	67	67
30	0	0	0

* Sera from individual mice were tested at the times indicated for antibody to growth hormone by ELISA. Sera with a titer ≥10 were considered positive. Each group contained 7–10 mice.

birth. The data in Table III show that antibody to GH, as measured by ELISA, developed in 100% of the mice inoculated at 3 and 14 d of age, whereas none of the mice inoculated at 30 d of age showed a rise in antibody to GH. Similarly, mice inoculated at 30 d of age failed to develop autoantibodies to insulin as measured by ELISA, nor to antigens in islet or pituitary cells as evaluated by immunofluorescence (data not shown).

In the ELISA studies described thus far, a peroxidase-labeled anti-mouse IgG was used. Evidence that reovirus also induces IgM autoantibodies comes from studies using a peroxidase-labeled antimouse IgM. In these experiments, 27 and 83% of the

mice, respectively, had IgM antibodies to insulin and GH at 10 d after infection. The IgM response was transient and only a few of the mice still had IgM autoantibodies at 14 and 21 d. In contrast, total serum IgG and IgM in reovirus-infected mice on days 14 and 21, as measured by radial immunodiffusion, were approximately two times higher than in uninfected controls.

Molecular Basis of Reovirus-induced Autoantibodies. Reovirus has a segmented genome with 10 different genes and the S1 gene codes for an outer capsid polypeptide (sigma 1) which is the hemagglutinin and the determinant of viral tropism (19, 20). In the experiments described thus far, reovirus type 1 was used. Preliminary studies in our laboratory showed that reovirus type 3 failed to induce autoantibodies to GH. To see if a specific segment of the viral genome was required for the induction of autoantibodies, recombinants between reovirus type 1 and type 3 were used. As seen in Fig. 8, reovirus type 1, but not reovirus type 3, induced autoantibodies to GH. When mice were infected with recombinant 3.HA1 (i.e., nine genes from reovirus type 3 and the S1 gene from reovirus type 1), all the mice developed autoantibodies to GH. In contrast, when mice were infected with recombinant 1.HA3 (i.e., nine genes from reovirus type 1 and the S1 gene from reovirus type 3), none of the mice developed autoantibodies to GH. Similarly, mice infected with clone 94 (i.e., a recombinant containing five genes from reovirus type 1 and five genes, including S1, from reovirus type 3) did not develop autoantibodies to GH. These experiments show that the S1 gene from reovirus type 1, which codes for the hemagglutinin, is required for the induction of autoantibodies to GH.

The final experiment was designed to study the relation between the S1 gene and the capacity of the virus to infect the pituitary. The data in Table IV, obtained by direct immunofluorescence, show that reovirus type 1 and recombinant 3.HA1 infected the anterior, but not the posterior or the intermediate pituitary. In contrast, reovirus type 3, recombinant 1.HA3 and recombinant clone 94 failed to infect the anterior pituitary. Minimal involvement was sometimes observed in the intermediate and posterior pituitary. As a control, the capacity of these viruses to infect neuronal and ependymal tissues (19, 20) was evaluated in the same group of animals. Reoviruses

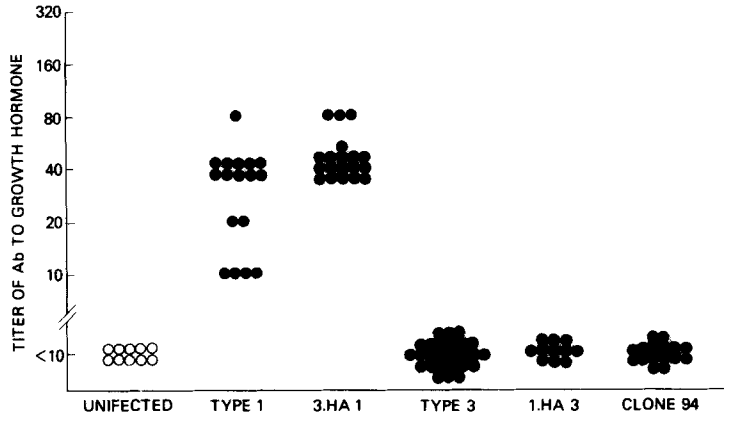

FIG. 8. Titer of autoantibody (Ab) to growth hormone, as determined by ELISA, in sera of mice infected with reovirus type 1, type 3, and recombinant viruses. Mice were inoculated intraperitoneally with the different viruses which had been passaged in L-929 cells and the animals were tested for antibody to growth hormone 14 d later. Each point represents an individual animal.

TABLE IV

Tissue Tropism of Recombinant Reoviruses as Evaluated by Immunofluorescence

Infecting virus*	Localization of viral antigens‡				
	Pituitary			Brain	
	Anterior	Intermediate	Posterior	Neurons	Ependyma
Type 1	++	−	−	−	+++
Recombinant 3.HA1	+++	−	−	+	+++
Type 3	−	+	−	+++	−
Recombinant 1.HA3	−	+	+	+++	−
Recombinant clone 94	−	−	−	+++	−

* Viruses were propagated in L-929 cells and mice were inoculated intracerebrally. Frozen sections of pituitary and brain obtained 7 d after infection were stained with FITC-labeled antisera to reovirus types 1 or 3.

‡ Approximately 10 pituitaries and 5 brains were examined from each of the virus-infected groups and at least 20 sections were prepared from each organ: (−) no fluorescence; (+) fluorescence in an occasional cell; (++) fluorescence in 5–20% of cells; (+++) fluorescence in >20% of cells.

having the S1 gene from type 1 infected ependymal cells, whereas reoviruses having the S1 gene from type 3 infected neuronal cells.

Discussion

By immunofluorescence, we found that mice infected with reovirus type 1 developed autoantibodies to cytoplasmic antigens in pancreatic islets, anterior pituitary, and gastric mucosa. Because these tissues contain hormone-producing cells and certain of these hormones are available in pure form, we were able to show by both absorption studies and by ELISA that sera from reovirus-infected animals contained autoantibodies to GH and insulin. A variety of controls demonstrated that reovirus infection, and not contamination of the viral inoculum with hormones or other tissue antigens, was responsible for the development of autoantibodies. Further evidence came from experiments with reovirus types 1 and 3 which demonstrated that only virus containing the type 1 hemagglutinin induced autoantibodies to GH. The possibility that reovirus induces autoantibodies to other hormones and host cell antigens, including surface antigens, is presently under investigation.

In addition to autoantibodies, many of the mice infected with reovirus type 1 developed polyendocrine disease. The diabetic picture was similar to that previously reported with reovirus type 3, except that alpha, beta, and delta cells were infected, rather than just beta cells (17). A possible explanation for the transient nature of the glucose abnormalities (Fig. 1) may be that beta cells from newborn mice regenerate faster than beta cells from older mice (31). Many of the mice also developed a runting syndrome characterized by retarded growth, oily hair, alopecia, and steatorrhea. Runting has been observed with reovirus type 3, but in <3% of the infected mice (32). In our experiments with reovirus type 1, 25–50% of the mice showed some signs of runting. The precise cause of virus-induced runting is not known (32, 33), but it is thought to be immunologically mediated because graft-vs.-host reactions also lead to runting (27, 28). The present study, however, suggests another possibility; that a GH deficiency, especially during the neonatal period, may at least contribute to the retarded growth component of the runting syndrome. This could result directly from

the replication of the virus and the destruction of infected cells in the anterior pituitary. Alternatively, virus-induced autoantibodies to hormones or to surface antigens on hormone-producing cells might result in a hormone deficiency by binding to hormones in the blood or by destroying hormone-producing cells (8, 34, 35). Deposition of immune complexes (e.g., hormone-antibody) also might lead to some of the manifestations of the runting syndrome (36). The relative contribution to the clinical picture of virus-induced cell destruction versus virus-induced autoimmune immunopathology remains to be clarified. Experiments in progress (T. Onodera, unpublished data) indicate that the severity of the hyperglycemia and runting can at least be partially reduced if animals are treated with immunosuppressive agents before infection.

Precisely how a virus infection causes the host to make antibodies against its own tissue is not known, but a variety of mechanisms have been proposed (9). One possibility is that the virus induces autoantibodies to certain antigens by interacting with cells containing those antigens. For example, the virus might insert, expose or modify antigens in the plasma membrane of the host cell (i.e., act as a hapten), or the infection might lead to the release of host antigens in precursor forms (e.g., pre-proinsulin, proinsulin, pre-GH) not ordinarily presented to the immune system (37–39). Alternatively, the virus might induce autoantibodies by lysing or activating subpopulations of lymphocytes (e.g., helper or suppressor cells) that regulate the immune response of the host (40, 41). Evidence that reovirus type 1 directly or indirectly affects the immune system comes from histopathology studies which indicate that at least in severly runted mice, there is some damage to the thymus and depletion of lymphocytes in the thymic-dependent areas of the spleen (T. Onodera, unpublished data).

With recombinant viruses, we have identified the segment of the viral genome required for the induction of autoantibodies to GH. These studies showed that the essential gene segment is S1, which codes for the hemagglutinin. The hemagglutinin binds to receptors on the surface of cells and thus determines the tropism of the virus. Weiner et al. (19, 20) showed that reoviruses containing the type 1 hemagglutinin infect ependymal cells, whereas reoviruses containing the type 3 hemagglutinin infect neurons. Our immunofluorescence studies confirm these observations and, in addition, show that viruses with the type 1 hemagglutinin, but not the type 3 hemagglutinin, infect cells in the anterior pituitary. Moreover, because only viruses with the type 1 hemagglutinin induce autoantibody to GH, this suggests that the interaction of virus with cells containing GH is required for the induction of autoantibody. Although Weiner et al. (42), recently reported that type 3, but not type 1, virus binds to murine lymphocytes, we cannot exclude the possibility that specific subpopulations of lymphocytes may have receptors for reovirus type 1 and that the interaction of virus with these lymphocytes may trigger autoantibodies. Thus, it is possible that infection of hormone-producing cells plus interaction of virus with cells of the immune system (e.g., polyclonal activation) may be required to initiate autoantibody production.

It is well known that after the initial onset of IDDM, many patients experience a remission, lasting from several weeks to months, and require little if any insulin. This remission or "honeymoon period" is followed by an exacerbation characterized by a permanent increase in the requirement for exogenous insulin (43). It has been suggested that the initial damage to beta cells may be caused by an environmental

insult (e.g., viruses, chemicals, drugs) (44) and that this is followed by a slower destructive process, perhaps due to autoimmune immunopathology (2, 6). The demonstration that reovirus can both damage hormone-producing cells and induce an autoimmune response lends credence to the hypothesis that diabetes mellitus and other diseases with an autoimmune component may sometimes be triggered by viral infections.

Summary

Mice infected with reovirus type 1 developed transient diabetes and a runting syndrome. The diabetes was characterized by hyperglycemia, abnormal glucose tolerance tests, and hypoinsulinemia. Inflammatory cells and viral antigens were found in the islets of Langerhans, and virus particles were seen in alpha, beta, and delta cells. The runting syndrome consisted of retarded growth, oily hair, alopecia, and steatorrhea. Inflammatory cells and viral antigens were found in the anterior, but not posterior pituitary. Electron microscopy revealed virus particles in growth hormone (GH)-producing cells and radioimmunoassay showed that the concentration of GH in the blood was decreased.

Examination of sera from infected mice revealed autoantibodies that, by immunofluorescence, reacted with cytoplasmic antigens in the islets of Langerhans, anterior pituitary, and gastric mucosa of uninfected mice. Absorption studies and enzyme-linked immunosorbent assays designed to identify the reactive antigens showed that some of the autoantibodies were directed against insulin and others against GH.

Reovirus type 3, in contrast to reovirus type 1, did not induce autoantibodies to GH. By use of recombinant viruses, the segment of the reovirus genome responsible for the induction of autoantibodies to GH was identified. Virus containing the S1 gene segment from reovirus type 1, which codes for the sigma 1 polypeptide (i.e., hemagglutinin), infected cells in the anterior pituitary and induced autoantibodies to GH, whereas virus containing the S1 gene segment from reovirus type 3 failed to infect cells in the anterior pituitary and did not induce autoantibodies to GH. We conclude that reovirus type 1 infection can lead to polyendocrinopathy and autoimmunity and that the S1 gene segment is required for the induction of autoantibodies to GH.

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