Immunomodulation of Encephalomyocarditis Virus-Induced Disease in A/J Mice

MICHAEL T. BARGER AND JOHN E. CRAIGHEAD*

Department of Pathology, University of Vermont, College of Medicine, Burlington, Vermont 05405

Received 28 June 1990/Accepted 24 January 1991

The E variant of encephalomyocarditis (EMC) virus causes an encephalomyelitis and coagulative necrosis of the pancreas and parotid glands in some but not all strains of inbred and outbred mice. In other models of disease caused by picornaviruses, depletion of specific lymphocyte subsets abrogates the development of tissue lesions. In this study, severe encephalomyelitis and acinar pancreatitis and parotitis developed in adult male A/J mice infected with 100 PFU of EMC virus. Depletion of the CD4⁺ subset of T lymphocytes in vivo with a monoclonal antibody (MAb) prior to EMC virus inoculation protects mice from developing encephalomyelitis, pancreatitis, and parotitis. This effect is also seen when animals are treated with anti-CD4 and anti-CD8 in combination, but the anti-CD8 MAb alone does not ameliorate the disease. Overall, concentrations of virus in tissues from anti-CD4-treated animals are lower than in immunologically intact control mice. Small-plaque variants of virus were also recovered from the tissues in some animals in this group. CD4⁺ lymphocytes are involved in the expression of EMC virus-induced pancreatitis and parotitis in A/J mice. This specific subset of T cells would appear to influence EMC viral tropism or replication in various organs.

Several members of the picornavirus family (group B coxsackievirus [CVB], foot-and-mouth disease virus, and encephalomyocarditis [EMC] virus) cause acute necrotizing pancreatitis and parotitis in mice (13, 20, 35). Mouse strain and gender influence susceptibility (1, 3, 7, 15, 16, 23, 26, 30, 33, 38, 39, 43, 44), and serotypically similar virus strains differ in their capacity to cause lesions (9, 12, 32, 36). The development of pancreatic and parotid acinar necrosis and inflammation has been described in adult male mice infected with a neurotropic variant (E) of EMC virus (8, 11, 40). The pathogenesis of these virus-induced changes is obscure, although immunopathogenic mechanisms are implicated in CVB-induced pancreatitis in DBA/2J mice (2).

This article describes the effect of T-lymphocyte subset depletion on the development of pancreatitis induced by the E variant of EMC virus in A/J mice. Anti-CD4 monoclonal antibody (MAb) administration prevents the development of the lesions and appears to attenuate the systemic virus infection. Thus, the development of EMC virus-induced pancreatitis and parotitis is modulated by the host immune system. Traditional T-lymphocyte-mediated immunity directed against pancreatic antigens is one possible pathogenic mechanism, but the appearance of the lesions only a few days after virus inoculation suggests alternative mechanisms. These possibilities are discussed.

MATERIALS AND METHODS

Mice. A/J mice, originally purchased from Jackson Laboratories, Bar Harbor, Maine, were bred and maintained in the animal care facilities in the University of Vermont Department of Pathology. Male mice 9 to 10 weeks old were caged in groups of four to six animals and maintained in a 12-h light–12-h dark cycle at 22°C with access to food and water ad libitum.

Virus and virus procedures. The E variant of EMC virus was originally isolated from the brain of an infected domestic pig in Panama and passed 11 times through mouse brains by

intracerebral injection (1138E) (10). Pools were made from diluted brain homogenate. One hundred PFU of virus was administered by intraperitoneal (i.p.) inoculation in 0.5 ml of Hanks's balanced salt solution.

Organs from mice killed by sodium pentobarbital overdose were bisected, and approximately half was stored at –70°C for virus titration. The organs were weighed, and the virus content of the tissue was determined by plaque assay on L-929 cell monolayers as described previously (9). The remainder of the tissue was fixed in neutral buffered Formalin and processed for histological examination.

Serum taken at the time of death was assayed for anti-EMC virus neutralization activity by the plaque reduction technique described previously (21). It was screened at a 1:20 dilution in Dulbecco's minimal essential medium (DMEM) (GIBCO) with 2% fetal bovine serum (GIBCO). The diluent had no virus neutralization activity.

MAb. MAbs GK1.5 and 2.43 recognizing murine CD4 (L3T4) and CD8 (Ly2.2) antigens, respectively, were prepared in mice with hybridomas originally obtained from the American Type Culture Collection as described previously (2, 21). MAbs were used in vivo to deplete animals of specific T-lymphocyte subsets. MAb as ascites fluid (1 mg diluted in a volume of 0.5 ml of phosphate-buffered saline [PBS]) was administered i.p. 48 and 24 h prior to virus inoculation. Depletion of lymphocyte subsets was confirmed by fluorocytometry on pooled lymph node or spleen cells collected from animals 72 h to 7 days after MAb administration as described previously (21). Approximately 90 to 95% CD4⁺ cells and 80 to 90% CD8⁺ cells were depleted from spleen, lymph node, and peripheral blood but not the thymus by this procedure (34). Repletion of CD4⁺ lymphocytes occurs gradually over a period of 2 to 3 months. An antilymphocyte MAb exhibited no EMC virus neutralizing activity.

Biochemical analyses. Blood glucose determinations were done with an Accu-Chek II Blood Glucose Monitor (Boehringer Mannheim Diagnostics, Indianapolis, Ind.) and Chem-strip bG on blood obtained from nonfasting mice by retro-

* Corresponding author.

TABLE 1. Effect of T-lymphocyte subset depletion on EMC virus infection

MAb treatment ^a	Max clinical grade ^b	No. dead/ no. tested	Histology score ^c		Serum amylase activity	
			Pancreas	Parotid	Maximum ^d (U/liter)	Elevation over baseline ^e
None (control)	4.5 ± 0.2	6/10	3.1 ± 0.3	3.1 ± 0.6	23,079 ± 2,769	15.4 ± 2.0
Anti-CD4 + anti-CD8	1.2 ± 0.7	1/10	0.5 ± 0.2 ^f	0.9 ± 0.7 ^g	4,186 ± 789 ^g	2.3 ± 0.4
Anti-CD4	1.8 ± 0.7	2/12	0.4 ± 0.2 ^f	0.8 ± 0.2 ^f	3,410 ± 560 ^g	2.4 ± 0.5
Anti-CD8	4.3 ± 0.3	6/12	3.1 ± 0.3	2.3 ± 0.6	32,019 ± 3,249 ^g	19.9 ± 2.3

^a MAb administered 24 and 48 h prior to EMC virus inoculation.

^b Clinical scoring is based on the degree of encephalitic signs on a scale of 0 to 5, with 0 being normal, 4 moribund, and 5 dead. Mean ± SEM.

^c Histology scoring is based on a scale from 0 to 4 as described in Materials and Methods. Mean ± SEM.

^d Maximum amylase activity is the mean of the peak activity for each mouse in each group. Mean ± SEM.

^e The elevation over baseline represents the peak amylase concentration as a multiple of the baseline level. Mean ± SEM.

^f $P < 0.01$ in comparison to control.

^g $P < 0.05$ in comparison to control.

orbital sinus puncture before and (in some experiments) on day 5 after virus inoculation.

Serum amylase was determined by a modification of the 4-nitrophenol maltoheptaoside chromogenic method (19, 25). Serum was collected before and at daily intervals after infection and stored at -70°C . Fifty microliters of a 1:50 dilution of serum in PBS were placed into a well of a 96-well flat-bottomed microtiter plate. The reaction was started by addition of 100 μl of amylase reagent (*para*-nitrophenol-maltoheptaoside substrate with α -glucosidase in saline buffer; Technicon Instruments Corp., Tarrytown, N.Y.), and the appearance of *para*-nitrophenol was monitored spectrophotometrically at 405 nm in an automated microplate reader (model EL309; Bio-Tek Instruments, Inc., Burlington, Vt.) over 20 min. Amylase activity was calculated by the rate of increase in optical density (19). Pancreatic tissue amylase was determined on suspensions of pancreatic tissue homogenate (10% in DMEM) diluted from 1:500 to 1:8,000 in PBS.

Histological evaluations. The parotid gland and pancreas were fixed briefly in Bouin's solution prior to overnight fixation in neutral buffered Formalin. After processing, 5- μm sections of tissue were stained with hematoxylin and eosin. Histological sections of pancreatic and parotid tissues were graded on the extent of necrosis and/or inflammation on a scale of 0 to 4: 0, no necrosis or inflammation; 1, few scattered focal lesions; 2, <50% of section involved; 3, >50% of section involved; 4, diffuse involvement.

Statistical analysis. Analysis of variance was used to test the differences between amylase concentrations in the different groups, with the Scheffe method used to analyze individual comparisons. The Kruskal-Wallis and Mann-Whitney tests were used to look at differences between the virus titers and histology scores.

RESULTS

EMC virus-induced pancreatitis and parotitis. EMC virus-infected mice developed signs of encephalomyelitis 3 to 5 days after inoculation; mortality was 60% by day 7 (Table 1). Infection was associated with a concomitant hyperamylasemia and depletion of amylase from the pancreas (Fig. 1). Glucose intolerance was not observed. Virus was detected in acinar tissues 24 h after infection, and maximal concentrations were found on days 6 to 7 (data not shown). Focal acinar necrosis and acute inflammation appeared in the pancreas and parotid glands by day 3, and diffuse necrosis and infiltration by mixed mononuclear cells were found on day 6 or 7 (Fig. 2A and D). The pancreatic ducts and islets of

Langerhans usually were not affected. A diffuse encephalomyelitis was consistently present in the central nervous system, but myocarditis was not observed. Serum virus neutralization antibody titers of 1:400 to 1:800 were demonstrated on day 5 after inoculation (data not shown).

Effect of T-lymphocyte subset depletion on induction of lesions. Signs of encephalomyelitis failed to develop in mice treated with the anti-CD4 MAb alone or in combination with the anti-CD8 MAb. Serum amylase concentrations increased slightly over the course of infection in animals administered either anti-CD4 or both anti-CD4 and anti-CD8 MAbs, but maximal serum amylase concentrations in individual animals exceeded five times the baseline level in only a single animal in three replicate experiments. Anti-CD8-treated mice developed disease comparable to control mice (PBS-treated group), and 50% of the anti-CD8-treated animals died. Hyperamylasemia occurred in the anti-CD8-treated and control mice to a similar extent, although amylase concentrations

FIG. 1. Amylase concentrations in serum and pancreas during EMC virus infection. Samples were collected before and daily after inoculation. Amylase activity was measured with a chromogenic substrate, *p*-nitrophenol maltoheptaoside. Each value is the mean of results for three animals ± SEM.

FIG. 2. Histopathology of the pancreas and parotid glands of EMC virus-infected mice. Pancreas (A, B, C) and parotid glands (D, E, F) were removed after death (day 6 or 7) and processed as described in the text. (A and D) Control group, no MAb treatment. (B and E) Anti-CD4 MAb treatment. (C and F) Anti-CD8 MAb treatment. Bar, 100 μ m. The sections were stained with hematoxylin and eosin.

tended to be higher in the animals treated with the anti-CD8 MAb (Fig. 3). The average mean increase in serum amylase concentrations for control infected animals was 15.4 and for anti-CD8-treated animals was 19.9 times the baseline value (Table 1).

Diffuse pancreatic acinar necrosis and inflammation devel-

oped in the control and anti-CD8-treated groups (Fig. 2). In contrast, the pancreas and parotid glands of the anti-CD4 MAb-treated animals and those animals administered both anti-CD4 and anti-CD8 either appeared normal or developed

FIG. 3. Effect of T-lymphocyte subset depletion on EMC virus-induced hyperamylasemia. Serum collected before and daily beginning on day 2 after inoculation was assayed for amylase activity as described in the text. Symbols: ●, control group; ▲, anti-CD4 MAb plus anti-CD8 MAb group; ○, anti-CD4 MAb group; ■, anti-CD8 MAb group. Mean amylase concentration $\pm$ SEM on days indicated.

FIG. 4. Effect of T-lymphocyte subset depletion on virus concentrations in tissue and virus neutralization antibody response. Organs were collected at death (day 6 or 7), and tissue homogenates were made. Each line joins the points that represent the virus concentrations of various organs from individual animals. Virus neutralization antibody activity at a 1:20 dilution of serum at death is recorded as positive (+) or negative (○). The result for individual mice is displayed adjacent to the point depicting the pancreatic virus titer.

FIG. 5. Effect of T-lymphocyte subset depletion on plaque morphology of virus from pancreas of EMC virus-infected mice. (A) Original inoculum used to infect animals. (B to E) Plates inoculated with pancreatic homogenates from infected animals: (B) PBS control, (C) dual MAb treatment, (D) anti-CD4 treatment, (E) anti-CD8 treatment.

only minimal focal areas of necrosis and inflammation (Fig. 2).

Virus concentrations in the pancreas and parotid gland were significantly reduced ($P < 0.05$) in animals treated with the anti-CD4 MAb or both anti-CD4 and anti-CD8 MAbs (Fig. 4). The amounts of virus in the hearts and brains were also decreased in comparison to controls ($P < 0.05$). Considerable variability in virus titers in tissue was noted among individual animals in the anti-CD4- and dual-antibody-treated mice. Virus concentrations in pancreatic tissue in several mice were similar to those in controls, whereas the amounts of virus in the tissue of the remaining animals were markedly reduced. Virus was recovered from at least one organ of every mouse, an indication that the MAb-treated mice consistently developed a systemic infection.

A serum neutralization antibody response was observed consistently at the time of death (day 6 or 7) (Fig. 4) in all control animals and mice treated with the anti-CD8 MAb. In contrast, the anti-CD4 MAb-treated mice, with markedly attenuated infections, failed to mount an antibody response. Animals with virus concentrations in tissue comparable to those in controls generally elaborated neutralization antibody detectable on the day of death.

The EMC virus inoculum produced plaques of variable size (0.5 mm to 5 mm) in L cells (Fig. 5A). The plaque morphology of virus recovered from the tissues of control animals was similar (Fig. 5B). Although it was a variable feature, the tissues of the anti-CD4-treated animals tended to yield small-plaque-producing virus variants (less than 2 mm) (Fig. 5B and C). Small and large (ca. 4 to 5 mm) variants were recovered by plaque purification from the animal tissue. Both variants proved to be pathogenic for mice and caused lesions of the pancreas and parotid glands. Preliminary data fail to indicate that the small-plaque variant replicates preferentially in anti-CD4 MAb-treated animals. We are currently isolating a series of variants in order to evaluate further the comparative pathogenicity of the different plaque size variants. We (12) and others (42) have described EMC virus plaque variants which differ in size and animal pathogenicity. The mechanisms involved, however, appear to be dissimilar.

DISCUSSION

Picornaviruses are small nonenveloped positive-strand RNA viruses that cause a variety of diseases in animals and humans. The pathogenesis of disease is generally thought to be consequent to the cytolytic effects of these viruses. Work from this laboratory implicates immunopathogenic mechanisms in animal models of CVB-induced myocarditis and pancreatitis (2) and EMC virus-induced diabetes mellitus (21). This prompted us to investigate the role of the immune system in EMC virus-induced pancreatitis and parotitis. Our results show that the $CD4^+$ subset of T lymphocytes affects not only the expression of disease but also virus replication and spread in tissues. EMC virus-induced pancreatitis and parotitis in A/J mice is prevented by depletion of $CD4^+$ lymphocytes in vivo with the anti-CD4 MAb. The protection afforded by $CD4^+$ lymphocyte depletion is also accompanied by both a reduction in virus concentrations in tissue and the apparent selection of small-plaque variants of the virus.

Depletion of specific T-lymphocyte subsets has been shown to protect mice from CVB-induced myocarditis (24, 29) and pancreatitis (2) and EMC virus strain M-caused necrosis of the islets of Langerhans and diabetes mellitus (21). In the EMC virus-induced acinar lesions, immunity against tissue antigens or virus-specific immunity might be of pathogenic importance; this possibility is not excluded by the data reported here, but it does not appear to account for all of our observations. Virus neutralization antibodies, one of the earliest primary immune responses, are initially detected on day 5 after infection, 1 or 2 days after the onset of disease. In view of the prompt appearance of acinar lesions after virus inoculation, the notion that the pathogenesis of EMC virus-induced pancreatitis and parotitis is mediated by traditional antigen-specific primary immune reactions must be questioned.

$CD4^+$ cells influence virus replication in target tissues. We found lower but variable virus concentrations in the organs of anti-CD4 MAb-treated animals that failed to develop pancreatitis and parotitis. $CD4^+$ lymphocytes do not serve as vectors or reservoirs for the virus, as assessed by infectious-center assays of lymphocytes recovered from infected animals 24 to 72 h after inoculation. Preliminary studies of the kinetics of EMC virus infection in anti-CD4 MAb-treated mice do not demonstrate substantial differences in the systemic patterns of virus replication in comparison to control animals (data not shown). The MAb effect does not appear to be a nonspecific response to the administration of immunoglobulin, since rat immunoglobulin G does not alter the course of the disease when administered i.p. prior to infection (36a). $CD4^+$ cells might participate passively in the infection by influencing, either directly or through cytokines, the expression of viral receptors or by inhibiting antiviral mechanisms. Preliminary data from our laboratory support such a contention. We have found that gamma interferon and tumor necrosis factor increase uptake of the viruses by transformed beta cells in vitro (37a). Presumably, cytokines acting similarly could be generated by $CD4^+$ lymphocytes during the early stages of infection.

Variability was noted in the antiviral neutralization activity of serum from anti-CD4 MAb-treated animals. Generally the animals in the group with the highest virus concentrations in tissue exhibited detectable antibody responses (Fig. 4). This observation suggests three possibilities. Infection may elicit $CD4^+$ T-cell-independent antibody responses in some animals, or the attenuated infection generates insufficient antigen to elicit a demonstrable antibody response.

Alternatively, anti-CD4 MAb treatment may have failed to eliminate or only transiently reduced CD4⁺ helper cell function in some animals. Whatever the explanation, antibody response was an independent variable, since pancreatic and parotid lesions consistently did not develop in the anti-CD4 MAb-treated mice.

Plaque size has been correlated with the virulence of some picornaviruses, but this is not a consistent property of members of this group of viruses (5, 6, 12, 17, 42). The virus recovered from animals treated with the anti-CD4 MAb, alone or with anti-CD8, tended to produce small plaques, whereas virus recovered from control and anti-CD8 MAb-treated animals had a plaque morphology similar to that of the original inoculum. Nonetheless, the pathogenicity of virus plaque-purified from pancreatic homogenates containing predominantly small-plaque variants exhibited a pathogenicity similar to that of the original inoculum (data not shown). Also, the virus recovered from animals inoculated with the small-plaque variant maintained its plaquing phenotype. These observations suggest that anti-CD4 MAb treatment did not select for nonpathogenic or avirulent viral variants. However, the presence of some "wild-type" virulent virus in the small-plaque pool cannot be ruled out.

Rhinovirus and poliovirus receptors are members of the immunoglobulin supergene family (18, 31, 37). Expression of these cell surface molecules can be modulated by cytokine products of lymphocytes and other cells of the immune system (4, 14, 27, 28, 41). Could EMC virus receptors be structurally similar to the identified picornavirus receptors, such as ICAM-1 for rhinoviruses (18, 37)? If so, EMC virus receptors might be induced or upregulated by CD4⁺ lymphocytes during the course of infection. Investigations are under way to determine the mechanism of protection induced by anti-CD4 MAb treatment in EMC-E virus-infected mice.

ACKNOWLEDGMENTS

We thank Sally Huber, Ram Sriram, and Mark Haynes for helpful discussions. We also thank Carol Hignite, Julie Cole, and Paul Tulikangas for excellent technical assistance and Hermilo Crisostomo, student research apprentice, who helped with some of the work presented here.

REFERENCES

- Babu, P. G., S. A. Huber, and J. E. Craighead. 1986. Contrasting features of the T-lymphocyte mediated diabetes in encephalomyocarditis virus-infected BALB/cBy and BALB/cCum mice. *Am. J. Pathol.* **124**:193-198.
- Blay, R., K. Simpson, K. Leslie, and S. Huber. 1989. Coxsackie virus-induced disease; CD4⁺ cells initiate both myocarditis and pancreatitis in DBA/2 mice. *Am. J. Pathol.* **135**:899-907.
- Boucher, D. W., K. Hayashi, J. Rosenthal, and A. L. Notkins. 1975. Virus-induced diabetes mellitus. III. Influence of the sex and strain of the host. *J. Infect. Dis.* **131**:462-466.
- Campbell, I. L., G. H. W. Wong, J. W. Schrader, and L. C. Harrison. 1985. Interferon-gamma enhances the expression of the major histocompatibility class I antigens on mouse pancreatic beta cells. *Diabetes* **34**:1205-1209.
- Cohen, J. I. 1989. Toward understanding the molecular basis for attenuation of picornaviruses. *Adv. Virus Res.* **36**:153-180.
- Cohen, S. H., R. K. Naviaux, K. M. Vanden Brink, and G. W. Jordan. 1988. Comparison of the nucleotide sequences of the diabetogenic and nondiabetogenic encephalomyocarditis virus. *Virology* **166**:603-607.
- Cook, S. H. S., R. M. Loria, and G. E. Madge. 1982. Host factors in coxsackievirus B4-induced pancreopathy. *Lab. Invest.* **46**:377-382.
- Craighead, J. E. 1965. Necrosis of the pancreas, parotid and lacrimal glands associated with encephalomyocarditis virus infection. *Nature (London)* **207**:1268-1269.
- Craighead, J. E. 1965. Some properties of the encephalomyocarditis, Columbia SK and Mengo viruses. *Proc. Soc. Exp. Biol. Med.* **119**:408-412.
- Craighead, J. E. 1966. Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus I. Myocardiotropic and neurotropic properties. *Am. J. Pathol.* **48**:333-345.
- Craighead, J. E. 1966. Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus. II. Lesions of the pancreas, parotid and lacrimal glands. *Am. J. Pathol.* **48**:375-386.
- Craighead, J. E. 1967. Pathogenic properties of encephalomyocarditis virus plaque variants. *Proc. Soc. Exp. Biol. Med.* **126**:806-813.
- Craighead, J. E. 1975. The role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus. *Prog. Med. Virol.* **19**:161-214.
- Dustin, M. L., and T. A. Springer. 1988. Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J. Cell Biol.* **107**:321-331.
- Friedman, S. B., L. J. Grotta, and L. A. Glasgow. 1972. Differential susceptibility of male and female mice to encephalomyocarditis virus: effects of castration, adrenalectomy, and the administration of sex hormones. *Infect. Immun.* **5**:637-644.
- Gaines, K. L., S. G. Kayes, and G. L. Wilson. 1986. Altered pathogenesis in encephalomyocarditis virus (D variant)-infected diabetes-susceptible and resistant strains of mice. *Diabetologia* **29**:313-320.
- Gould, C. L., M. L. Trombley, N. J. Bigley, K. G. McMannama, and D. J. Giron. 1984. Replication of the diabetogenic and nondiabetogenic variants of encephalomyocarditis (EMC) virus in ICR swiss mice. *Proc. Soc. Exp. Biol. Med.* **175**:449-453.
- Greve, J. M., G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, and A. McClelland. 1989. The major human rhinovirus receptor is ICAM-1. *Cell* **56**:839-847.
- Hägele, E., E. Schaich, E. Rauscher, P. Lehman, H. Bürk, and A. Wahlfeld. 1982. Mechanism of action of human pancreatic and salivary α -amylase on α -4-nitrophenyl maltoheptaoside substrate. *Clin. Chem.* **28**:2201-2205.
- Harrison, A. K., S. P. Bauer, and F. A. Murphy. 1972. Viral pancreatitis: ultrastructural pathological effects of coxsackie B3 infection in newborn mouse pancreas. *Exp. Mol. Pathol.* **17**:206-219.
- Haynes, M. K., S. A. Huber, and J. E. Craighead. 1987. Helper-inducer T-lymphocytes mediate diabetes in EMC-infected BALB/cByJ mice. *Diabetes* **36**:877-881.
- Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol. 1982. Rapid evolution of RNA genomes. *Science* **215**:1577-1585.
- Huber, S. A., P. G. Babu, and J. E. Craighead. 1985. Genetic influences on the immunologic pathogenesis of encephalomyocarditis (EMC) virus-induced diabetes mellitus. *Diabetes* **34**:1186-1190.
- Huber, S. A., and P. A. Lodge. 1986. Coxsackievirus B-3 myocarditis: identification of different pathogenic mechanisms in DBA/2 and BALB/c mice. *Am. J. Pathol.* **122**:284-291.
- Jansen, A. P., and P. G. A. B. Wydeveld. 1958. α -(p-Nitrophenyl) maltoside as a substrate for the assay of amylase. *Nature (London)* **182**:525-526.
- Kroman, H., Å. Lernmark, B. F. Vestergaard, J. Egeberg, and J. Nerup. 1979. The influence of the major histocompatibility complex (H-2) on experimental diabetes in mice. *Diabetologia* **16**:107-114.
- Leiter, E. H., G. J. Christianson, D. V. Serreze, A. T. Ting, and S. M. Worthen. 1989. MHC antigen induction by interferon gamma on cultured mouse pancreatic B cells and macrophages. *J. Exp. Med.* **179**:1243-1262.
- Leszczynski, D. 1990. Interleukin-1 alpha inhibits the effects of gamma-interferon and tumor necrosis factor alpha on the expression of the major histocompatibility antigens by the rat endothelium. *Am. J. Pathol.* **136**:229-237.

29. Lodge, P. A., M. Herzum, J. Olszewski, and S. A. Huber. 1987. Coxsackievirus B-3 myocarditis: acute and chronic forms of the disease caused by different immunopathogenic mechanisms. *Am. J. Pathol.* **128**:455-463.
30. Loria, R. M., L. B. Montgomery, L. A. Corey, and V. M. Chinchilli. 1984. Influence of diabetes mellitus heredity on susceptibility to coxsackievirus B4. *Arch. Virol.* **81**:251-262.
31. Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello. 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* **56**:855-865.
32. Murnane, T. G. 1982. Encephalomyocarditis virus, p. 353-357. In H. L. Foster, J. D. Small, and J. G. Fox (ed.), *The mouse in biomedical research*, vol. II. Academic Press, Inc., New York.
33. Onodera, T., J. Yoon, K. S. Brown, and A. L. Notkins. 1978. Evidence for a single locus controlling susceptibility to virus-induced diabetes mellitus. *Nature (London)* **274**:693-696.
34. Ranges, G. E., S. M. Cooper, and S. Sriram. 1987. In vivo immunomodulation by monoclonal anti-L3T4. 1. Effects on humoral and cell-mediated immune response. *Cell. Immunol.* **106**:163-173.
35. Ross, M. E., K. Hayashi, and A. L. Notkins. 1974. Virus-induced pancreatic disease: alterations in concentration of glucose and amylase in blood. *J. Infect. Dis.* **129**:669-676.
36. Ross, M. E., T. Onodera, K. Hayashi, and A. L. Notkins. 1975. Virus-induced diabetes mellitus. V. Biological differences between the M variant and other strains of encephalomyocarditis virus. *Infect. Immun.* **12**:1224-1226.
- 36a. Sriram, S. Personal communication.
37. Staunton, D. E., V. J. Merluzzi, R. Rothlein, R. Barton, S. D. Marlin, and T. M. Springer. 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* **56**:849-853.
- 37a. Topham, D. Personal communication.
38. Webb, S. R., R. M. Loria, G. E. Madge, and S. Kibrick. 1976. Susceptibility of mice to group B coxsackie virus is influenced by the diabetic gene. *J. Exp. Med.* **143**:1239-1248.
39. Webb, S. R., and G. E. Madge. 1980. The role of host genetics in the pathogenesis of coxsackievirus infection in the pancreas of mice. *J. Infect. Dis.* **141**:47-54.
40. Wellman, K. F., B. W. Volk, P. Brancato, and D. Amsterdam. 1973. Encephalomyocarditis virus (E variant) in mice. *Arch. Pathol.* **95**:304-331.
41. Wuthrich, R. P., A. M. Jevnikar, F. Takei, L. H. Glimcher, and V. E. Kelley. 1990. Intercellular adhesion molecule-1 (ICAM-1) expression is upregulated in autoimmune murine lupus nephritis. *Am. J. Pathol.* **136**:441-450.
42. Yoon, J., P. R. McClintock, T. Onodera, and A. L. Notkins. 1980. Virus-induced diabetes mellitus. XVIII. Inhibition by a nondiabetogenic variant of encephalomyocarditis virus. *J. Exp. Med.* **152**:878-892.
43. Yoon, J., and A. L. Notkins. 1976. Virus-induced diabetes mellitus. VI. Genetically determined host differences in the replication of encephalomyocarditis virus in pancreatic beta cells. *J. Exp. Med.* **143**:1170-1185.
44. Yoon, J., T. Onodera, and A. L. Notkins. 1977. Virus-induced diabetes mellitus. VIII. Passage of encephalomyocarditis virus and severity of diabetes in susceptible and resistant strains of mice. *J. Gen. Virol.* **37**:225-232.