Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10252–10256, November 1991
Medical Sciences

Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin

(diabetes/tolerance/autoimmunity/immunotherapy/insulin)

Z. JENNY ZHANG*, LAURIE DAVIDSON†, GEORGE EISENBARTH†, AND HOWARD L. WEINER*

*Brigham and Women's Hospital and †Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115

Communicated by Paul E. Lacy, August 2, 1991

ABSTRACT **Nonobese diabetic (NOD) mice spontaneously develop an autoimmune form of diabetes associated with insulitis. A number of immunomodulatory therapies have been investigated as a treatment for the disease process. Oral administration of the autoantigens myelin basic protein and collagen type II suppresses experimental models of encephalomyelitis and arthritis. We have now found that oral administration of insulin delays the onset and reduces the incidence of diabetes in NOD mice over a 1-year period in animals administered 1 mg of porcine insulin orally twice a week for 5 weeks and then weekly until 1 year of age. As expected, orally administered insulin had no metabolic effect on blood glucose levels. The severity of lymphocytic infiltration of pancreatic islets was also reduced by oral administration of insulin. Furthermore, splenic T cells from animals orally treated with insulin adoptively transfer protection against diabetes, demonstrating that oral insulin administration generates active cellular mechanisms that suppress disease. These results show that oral insulin affects diabetes and the pancreatic cellular inflammatory process in the NOD mouse and raise the possibility that oral administration of insulin or other pancreatic autoantigens may provide a new approach for the treatment of autoimmune diabetes.**

Type I diabetes or insulin-dependent diabetes mellitus (IDDM) is thought to be an autoimmune disease in humans (1–3). The nonobese diabetic (NOD) mouse spontaneously develops IDDM that has many immunological and pathological similarities to human insulin-dependent diabetes. The autoimmune nature of the disease is suggested by the lymphocytic infiltration of the islets of Langerhans, which precedes the destruction of insulin-producing beta cells (4). As such, the NOD mouse has served as one of the primary models for IDDM and a model in which new approaches for immunotherapy have been investigated.

A variety of immunomodulatory treatments have been studied in the NOD mouse. In general, treatments that affect T-cell function or are immunosuppressive have been effective, such as neonatal thymectomy and *in vivo* treatment with anti-CD4 monoclonal antibody and cyclosporine A (5–7). A major impetus behind such studies has been to develop approaches that may be utilized to treat human IDDM. Clinical trials in humans have demonstrated that antigen nonspecific immunosuppression with drugs such as cyclosporine A and azathioprine can affect beta-cell destruction after diabetes onset, but such therapy is not curative and is associated with drug-related toxicities (8, 9). The ability to identify patient populations at risk for diabetes (10, 11) makes the development of disease-specific nontoxic forms of therapy that can be administered to prediabetics to prevent or reduce the incidence of diabetes a major therapeutic goal.

We have been investigating antigen-driven peripheral immune tolerance as a means to suppress autoimmune processes, using the oral route of antigen exposure. Orally administered antigen stimulates the immune system in a physiologic fashion and has long been recognized to produce systematic immunologic hyporesponsiveness or tolerance (12–14). We and others have found that oral administration of autoantigens suppresses animal models of autoimmunity including experimental autoimmune encephalomyelitis (EAE) (15–17), adjuvant- and collagen-induced arthritis (18–20), and experimental autoimmune uveitis (21). Oral tolerance as a means to treat diabetes is especially attractive because of its virtual lack of toxicity and its inherent clinical applicability. In addition, such therapy could be applicable to pancreatic islet transplantation. In the present report, we have found suppression of diabetes in the NOD mouse by oral administration of insulin.

MATERIALS AND METHODS

Animals. NOD mice were purchased from Taconic Farms, maintained in our animal facility, and fed regularly with Purina Mouse Chow 5015 or 5008. The animals studied in experiments in Table 1 and Fig. 4 were housed in a conventional room, and those studied in all other experiments were housed in a virus antibody-free (VAF) facility. Female NOD mice were used for all experiments except for recipients in adoptive transfer experiments.

Assessment of Diabetes. Mice were monitored for development of diabetes weekly by urinary glucose testing with test strips (Eli Lilly). Glycosuric mice were then bled to check for glycemia by using a glucose analyzer (Beckman). Diabetes was confirmed by hyperglycemia (>13.8 mM) for 2 consecutive weeks.

Antigens. Porcine monocomponent insulin was purchased from Novo Biolabs (Danbury, CT). Myelin basic protein (MBP) was prepared as described (15).

Oral Administration of Antigen. Insulin or MBP in phosphate-buffered saline (PBS; 1.7 mM KH_2PO_4/5 mM Na_2HPO_4/150 mM NaCl) was administered to mice orally through a syringe fitted with a ball-type feeding needle in a volume of 0.5 ml per mouse per feeding.

Histopathology. The animals were sacrificed by cervical dislocation, and the pancreases were taken and immediately frozen. Cryosections (3 or 4 sections per mouse) were fixed with acetone and double-stained with (*i*) biotinylated monoclonal anti-thy-1.2 antibody plus avidin–peroxidase conjugate and (*ii*) monoclonal anti-beta-cell antibody (A2B5) plus alkaline phosphatase-conjugated anti-mouse IgM. The degree of insulitis was scored blindly by two independent observers using a semiquantitative scale ranging from 0 to 4: 0, normal islet with no sign of T-cell infiltration; 1, focal peri-islet T-cell infiltration; 2, more extensive peri-islet infil-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NOD, nonobese diabetic; IDDM, insulin-dependent diabetes mellitus; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; VAF, virus antibody-free.

tration but with lymphocytes less than one-third of the islet area; 3, intraislet T-cell infiltration in one-third to one-half of the islet area; 4, extensive intraislet inflammation involving more than half of the islet area.

Adoptive Transfer of Diabetes and T-Cell Depletion. The adoptive transfer experiments were carried out by the method of Wicker *et al.* (22) with slight modifications. Briefly, donor splenocytes were prepared from newly diabetic female animals (diagnosed within 14 days), resuspended in Hanks' balanced salt solution (HBSS), and injected i.v. through the retroorbital plexus (1×10^7 cells per recipient) to 7-week-old male NOD mice, which were irradiated with 770 R from a ^{137}Cs source 24 hr prior to the transfer. Five-million modulator cells from insulin-fed or control-fed animals were cotransferred with splenocytes from newly diabetic animals into male recipients. For T-cell depletion, splenocytes from insulin-fed animals were incubated with anti-thy-1.2 monoclonal antibody (diluted 1:200; from Accurate Chemicals, Westbury, NY) at a concentration of 2×10^7 cells per ml, at room temperature for 60 min, followed by an incubation with Low-Tox rabbit complement (1:15; Cedarlane Laboratories, Hornby, ON, Canada) for 30 min at 37°C. Control cells were treated with complement alone. Cells were washed three times with HBSS prior to transfer. Five-million anti-thy-1.2- or complement-treated cells were cotransferred.

RESULTS AND DISCUSSION

A number of autoantigens have been identified as potential target antigens of an autoimmune attack that leads to the development of diabetes. These include insulin, glutamic acid decarboxylase (GAD), carboxypeptidase H, insulin secretory granule proteins, and heat shock proteins (23–25). To test the effect of oral administration of insulin on the development of diabetes, female NOD mice at 5 weeks of age were fed PBS or 10 μg, 100 μg, or 1 mg of porcine insulin twice weekly for 5 weeks and then weekly until the animals reached 1 year of age. There was a marked delay in the onset and a decreased incidence of diabetes in animals fed 1 mg of porcine insulin (Table 1; Fig. 1, $P = 0.02$, Kaplan–Meier analysis) with a slight effect at 100 μg. Note that the incidence of diabetes in the control group was relatively low. This may be related to the frequent handling of the animals associated with feeding and to the housing of the animals for this experiment in a non-VAF facility. To test the effect of oral insulin in animals with a higher incidence of diabetes, a second experiment was conducted in a VAF facility. In addition, a group of animals was also fed 1 mg of MBP as a control antigen. A decreased incidence of diabetes following oral insulin was observed, although the overall incidence of diabetes was higher. Specifically, the incidence of diabetes in animals at 30 weeks was as follows: 13 of 30 fed PBS, 14 of 30 fed MBP, 13 of 29 fed 10 μg of insulin, 10 of 30 fed 100 μg of insulin, and 6 of 30 fed 1 mg of insulin ($P < 0.05$ for animals fed 1 mg of insulin vs. control and animals fed MBP).

Table 1. Suppression of IDDM in NOD mice by oral administration of porcine insulin

Feeding treatment	Diabetes incidence, %		
	6 months	9 months	12 months
Control (PBS)	20.5	44.1	49.2
Insulin in PBS			
10 μg	16.7	23.8	37.3
100 μg	11.1	28.5	43.8
1 mg	0*	8.0*	26.4†

Five-week-old female NOD mice (27–30 per group) were fed with various dosages of porcine insulin in PBS (control group received PBS alone) twice weekly for 5 weeks and weekly until 1 year of age. Beginning at 12 weeks of age, the mice were examined weekly for diabetes.

*$P \leq 0.01$ (compared with control).

†$P = 0.02$ (compared with control).

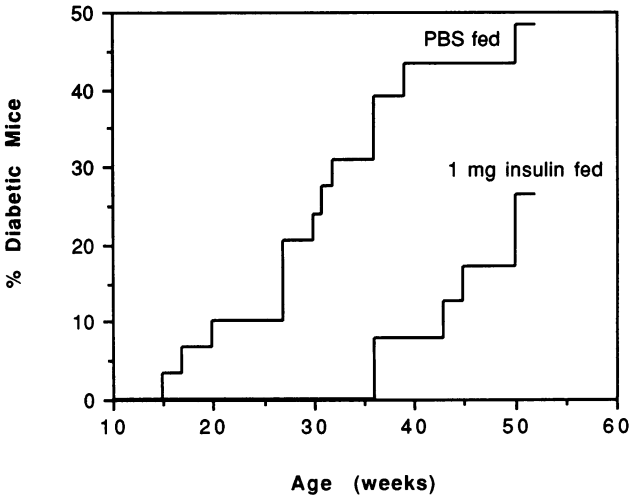

FIG. 1. Effect of oral administration of porcine insulin on IDDM in female NOD mice. Life table analysis of the control group and the group fed 1 mg of insulin from Table 1 ($P = 0.02$, Kaplan–Meier analysis).

It has been reported (26) that low doses of subcutaneous insulin may affect the onset of diabetes in NOD mice. Orally administered insulin is not metabolically active, presumably because it is degraded in the stomach. Degradation of proteins in the gastrointestinal tract does not affect oral tolerance and actually may facilitate orally induced tolerance by creating small protein fragments that are better able to interact with gut-associated lymphoid tissue (27). Nonetheless, to determine whether any metabolic effects could be discerned in animals being fed 1 mg of insulin, blood glucose levels were measured in 17-week-old animals. The average blood glucose prior to the weekly insulin feeding was 7.56 mM in animals fed PBS and 7.53 mM in animals fed 1 mg of insulin. Thirty minutes after feeding, the blood glucose in animals fed PBS

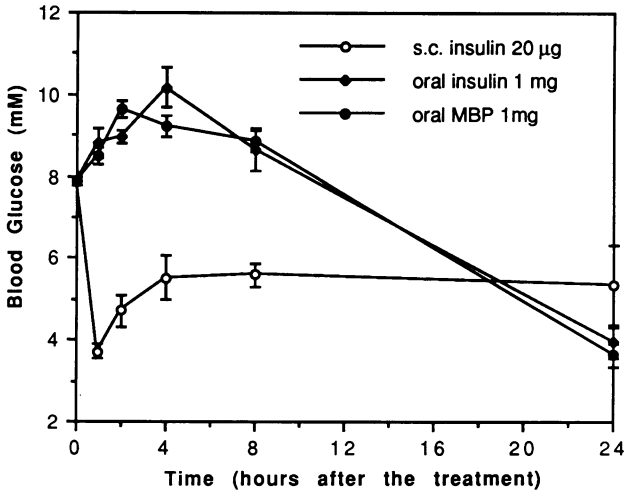

FIG. 2. Effect of oral insulin on blood glucose. Seven-week-old female NOD mice (25 mice per group) were treated orally with 1 mg of porcine insulin or 1 mg of guinea pig MBP or were injected subcutaneously with 20 μg of porcine insulin. All mice were bled before treatment, and 5 mice from each group were bled again 1, 2, 4, 8, and 24 hr after treatment. Individual plasma samples were measured in duplicate for glucose levels by using a Beckman glucose analyzer that was standardized three times during the entire analysis. Data represent mean value ± standard error.

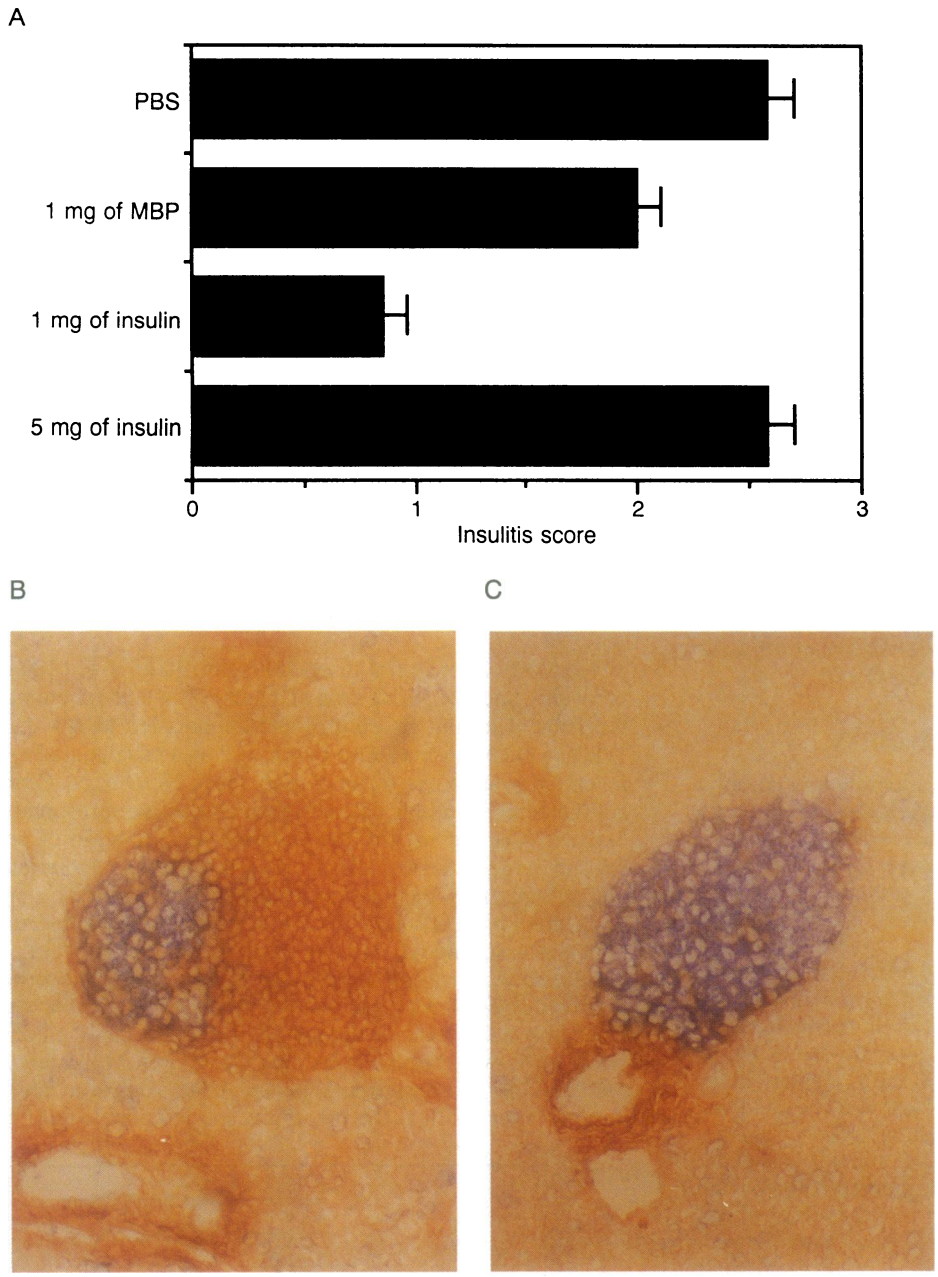

FIG. 3. Effect of feeding porcine insulin on insulitis in NOD mice. Five-week-old female NOD mice (8–10 mice per group) were fed PBS, 1 mg or 5 mg of insulin in PBS, or 1 mg of MBP in PBS twice weekly for 5 weeks. At 10 weeks of age, the animals were sacrificed, and pancreases were taken for histopathological examinations. Eight to 12 islets from each animal were scored. (*A*) Insulitis score. Data are expressed as the mean score of each group ± SEM ($P < 0.001$ for group fed 1 mg of insulin vs. group fed PBS or 1 mg of MBP). (*B*) Representative islet from control animal with pronounced lymphocyte infiltration (histopathologic score = 4). (*C*) Representative islet from animal fed 1 mg of insulin with minimal inflammation (histopathologic score = 1).

was 8.53 mM and in animals fed 1 mg of insulin was 8.63 mM. In an additional study, 7-week-old NOD mice were fed 1 mg of insulin or 1 mg of MBP. All animals were kept in a fasting state. Animals given 20 μg of subcutaneous insulin had an immediate drop in blood glucose (Fig. 2). In animals fed 1 mg of insulin or 1 mg of MBP, an increase in blood glucose was observed, perhaps related to the stress of gastric intubation, followed by a decrease in blood glucose 8 hr later as the animals were in a fasting state. Note that animals fed 1 mg of insulin for 1 year responded normally to subcutaneous insulin (data not shown). These results show that oral insulin has no metabolic effect on blood glucose either acutely or chronically.

To determine whether feeding insulin affected lymphocytic infiltration of pancreatic islets, animals in a separate series of experiments were fed 1 mg of insulin twice weekly for 5 weeks and were sacrificed at 10 weeks of age and examined for insulitis. There was a marked reduction of insulitis in animals fed 1 mg of insulin vs. those fed 1 mg of MBP (Fig. 3; 0.85 ± 0.1 vs. 1.99 ± 0.1; $P < 0.01$). Note that feeding 5 mg of insulin did not affect insulitis. A similar dose–response effect has been observed with oral tolerization to collagen in animal models of adjuvant and collagen-induced arthritis in which the suppressive effect of oral collagen was lost with increased doses (18–20). We also have observed a loss of suppression of EAE in the SJL mouse by orally administered MBP with increasing doses (28).

The majority of studies on the mechanism of oral tolerance report that active cellular suppression occurs (14). We have adoptively transferred disease protection with lymphocytes from fed animals in both the EAE and adjuvant arthritis models (16–18). To investigate whether active cellular mechanisms were associated with suppression of diabetes in the NOD mouse after oral administration of insulin, an accelerated diabetes model was utilized, in which diabetes is accelerated in young NOD mice by adoptive transfer of splenocytes from diabetic NOD donors; this provides a sensitive and more rapid assay for investigating immunomodulation of disease. Spleen cells from animals fed 1 mg of insulin, PBS, or 1 mg of MBP 5 times over 2 weeks were cotransferred with spleen cells from diabetic animals. Accelerated diabetes in NOD mice was suppressed by splenocytes from insulin-fed but not PBS- or MBP-fed animals (Fig. 4; $P = 0.037$, "logrank" test for all groups).

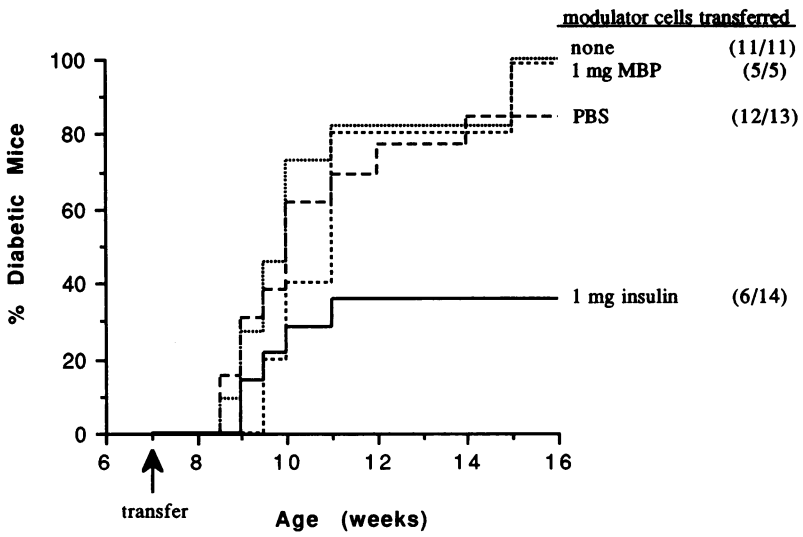

FIG. 4. Suppression of adoptively transferred diabetes by splenocytes from female NOD mice fed insulin. Modulator cells were freshly obtained from 6-week-old female NOD mice that had been fed 1 mg of insulin, 1 mg of MBP, or PBS five times over the previous 2 weeks. Ten million splenocytes isolated from female diabetic NOD mice were cotransferred with 5 million modulator cells from fed animals to 7-week-old syngeneic male recipients that had been irradiated with 770 R 24 hr earlier. The onset of diabetes in the recipients was checked twice weekly by assaying for glycosuria and confirmed by presence of hyperglycemia (>13.8 mM). $P = 0.037$ (logrank test) for all groups; $P = 0.021$ for animals fed insulin vs. those fed PBS.

To determine whether the suppression was T cell dependent, T cells were depleted from splenocytes of insulin-fed animals prior to adoptive transfer. Eight weeks after transfer, the incidence of accelerated diabetes in animals receiving no modulators was 10/11; in animals receiving complement-alone-treated modulators from insulin-fed animals was 2/10, and in animals receiving T cell-depleted modulators from insulin-fed animals was 9/10 ($P = 0.02$). Others have reported suppression of accelerated disease with transfer of 20×10^6 spleen cells from nondiabetic 8-week-old animals (29). We did not observe protection by spleen cells from control animals with 5×10^6 cells transferred.

The effects we observed in the NOD mouse are not related to nonspecific suppressive effects of orally administered insulin as oral administration of 1 mg of insulin had no effect on the development of EAE in the SJL mouse or on cellular proliferative response to concanavalin A or lipopolysaccharide (data not shown). In other studies of oral tolerance in autoimmune models, we also found disease protection to be antigen and disease specific. Thus, the antigens we have used for oral tolerization, MBP, collagen type II, and S antigen suppress EAE, adjuvant arthritis, and experimental uveitis, respectively, without affecting the other diseases. Species-specific autoantigens are not a requisite to induce oral tolerance as we have found suppression of EAE in the Lewis rat with bovine MBP.

Although we have shown suppression of diabetes and insulitis in the NOD mouse by oral administration of insulin, the role of autoimmunity to insulin in the development of diabetes in the NOD mouse and in man remains to be defined. Anti-insulin antibodies are found in both NOD mice and patients with type I diabetes (30). In patients, anti-insulin autoantibodies can be found prior to the onset of insulin therapy, are HLA-DR4-associated, and are correlated with the rate of disease progression (31, 32). Cellular reactivity to insulin occurs in man and has been reported to be of increased frequency in prediabetic individuals (33). Cellular immunity to insulin has not been extensively studied in the NOD mouse, and in initial experiments we have not found cellular immune responses to insulin as measured by thymidine incorporation in the spleen or lymph nodes of NOD mice, though further investigations are required in this area using more sensitive assays and studying cells isolated from the pancreas.

Adoptive transfer experiments demonstrate that transferable active suppression of diabetes in the NOD mouse by splenic T cells is generated by oral administration of insulin. Recent studies from our laboratory suggest that the T cells that adoptively transfer suppression of experimental autoimmune encephalomyelitis following oral administration of MBP are triggered in an antigen-specific fashion but mediate their effect by the release of the antigen-nonspecific suppressor cytokine transforming growth factor β (TGF-β) in close proximity to effector cells (34). We have termed this mechanism "antigen-driven bystander suppression" (35). Thus, it is possible that insulin is not a pathogenic autoantigen in the NOD mouse but that the regulatory cells generated in the gut by feeding insulin migrate to the pancreas and are triggered by insulin to release TGF-β, which down-regulates the local inflammatory processes in the pancreas. Further investigations are required to determine whether oral administration of insulin affects diabetes in the NOD mouse by suppression of anti-insulin autoimmunity or by the aforementioned antigen-driven bystander suppression mechanism.

It remains to be determined whether oral administration of other islet cell-specific antigens such as glutamic acid decarboxylase, carboxypeptidase H, heat shock proteins, or secretory granule proteins can also suppress diabetes in the NOD mouse. For application to human disease states, we have found that oral administration of autoantigens suppresses both established EAE and adjuvant arthritis, demonstrating the ability to effect an ongoing immune response (18, 36).

Although our data clearly demonstrate amelioration of diabetes in the NOD mouse by oral administration of insulin, protection is not complete. We have observed that adjuvants such as lipopolysaccharide, when given orally, enhance the protective effects of oral tolerance to MBP in the EAE model (37). In addition, after week 10 of the NOD mouse, insulin was administered once per week. Thus, the use of tolerogenic adjuvants to enhance suppression, or more frequent dosing schedules, may lead to more complete prevention of diabetes. Additionally, it may be that oral administration of more than one or a different pancreatic target antigen will further enhance protection.

One of the primary goals for the immunotherapy of autoimmune diseases is to find nontoxic antigen-specific therapies that can be administered early in the course of the disease. Our results in the NOD mouse model of diabetes raise the possibility that orally administered insulin and/or other pancreatic antigens could provide a new approach for the prevention and treatment of autoimmune diabetes in man.

We thank Ms. Nancy S. Blogg for her technical support. This work was supported by National Institutes of Health Grants 2P30DE36836 and DK32083 to the Joslin Diabetes Center and by Autoimmune, Inc.

1. Castano, L. & Eisenbarth, G. S. (1990) *Annu. Rev. Immunol.* **8**, 647–679.

2. Rossini, A., Mordes, J. P. & Greiner, D. L. (1989) *Curr. Opinion Immunol.* **2,** 598–603.
3. Acha-Orbea, H. & McDevitt, H. O. (1990) *Curr. Top. Microbiol. Immunol.* **156,** 103–119.
4. Leiter, E. H., Serreze, D. V. & Prochaz, K. A. M. (1990) *Immunol. Today* **11,** 147–149.
5. Ogawa, M., Maruyama, T., Hasegawa, T., Kanaya, T., Kobayash, F., Tochino, Y. & Uda, H. (1985) *Biomed. Res.* **6,** 103–105.
6. Mori, Y., Suko, M., Okudiara, H., Matsuba, I., Tsuruoka, S., Sasaki, A., Yokoyama, H., Tanase, T., Shida, T., Nishimura, M., Terada, E. & Ikeda, Y. (1986) *Diabetologia* **29,** 244–247.
7. Shizuru, A., Edwards-Taylor, C., Banks, B. A., Gregory, A. K. & Fathman, C. G. (1988) *Science* **240,** 659–662.
8. Silverstein, J., Maclaren, N., Riley, W., Spillar, R., Radjenovic, D. & Johnson, S. (1988) *N. Engl. J. Med.* **319,** 599–604.
9. Bougneres, P. F., Landais, P., Boisson, C., Carel, J. C., Frament, N., Boitard, C., Chaussain, J. L. & Bach, J. F. (1990) *Diabetes* **39,** 1264–1272.
10. Ziegler, A. G., Herskowitz, R. D., Jackson, R. A., Soeldner, J. S. & Eisenbarth, G. S. (1990) *Diabetes Care* **13,** 762–775.
11. Bonifacio, E., Bingley, P. J., Shattock, M., Dean, B. M., Dunger, D., Gale, E. A. M. & Bottazzo, G. F. (1990) *Lancet* **335,** 147–149.
12. Wells, H. G. (1911) *J. Infect. Dis.* **9,** 147–171.
13. Chase, M. (1946) *Proc. Soc. Exp. Biol. Med.* **61,** 257–259.
14. Mowat, A. M. (1987) *Immunol. Today* **8,** 93–98.
15. Higgins, P. J. & Weiner, H. L. (1988) *J. Immunol.* **140,** 440–445.
16. Lider, O., Santos, L. M. B., Lee, C. S. Y., Higgins, P. J. & Weiner, H. L. (1989) *J. Immunol.* **142,** 748–752.
17. Bitar, D. M. & Whitacre, C. C. (1988) *Cell. Immunol.* **112,** 364–370.
18. Zhang, J. Z., Lee, C. S. Y., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **145,** 2489–2493.
19. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, F. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7443–7446.
20. Thompson, H. S. G. & Staines, N. A. (1986) *Clin. Exp. Immunol.* **64,** 581–586.
21. Nussenblatt, R. B., Caspi, R., Mahdi, R., Chan, C., Roberge, F., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **144,** 1689–1695.
22. Wicker, L. S., Miller, B. J. & Mullen, Y. (1986) *Diabetes* **35,** 855–860.
23. Baekkeskov, S., Aanstoot, H., Christgau, S., Reetz, A., Solimena, M., Cascalho, M., Folli, F., Richter-Olesen, H. & Camilli, P. (1990) *Nature (London)* **347,** 151–156.
24. Roep, B. O., Arden, S. D., de Vries, R. R. P. & Hutton, J. C. (1990) *Nature (London)* **345,** 632–634.
25. Elias, D., Markovits, D., Reshef, T., van der Zee, R. & Cohen, I. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1576–1580.
26. Atkinson, M. A., Maclaren, N. K. & Luchetta, R. (1990) *Diabetes* **39,** 933–937.
27. Michael, J. G. (1989) *Immunol. Invest.* **18,** 1049–1054.
28. Al-Sabbagh, A. & Weiner, H. L. (1991) *Neurology* **41,** 318 (abstr.).
29. Boitard, C., Yasunami, R., Dardenne, M. & Bach, J. F. (1989) *J. Exp. Med.* **169,** 1669–1680.
30. Ziegler, A. G., Vardi, P., Ricker, A. T., Hattori, M., Soeldner, J. S. & Eisenbarth, G. S. (1989) *Diabetes* **38,** 358–363.
31. Ziegler, R., Alper, C. A., Awdeh, Z. L., Castano, L., Brink, S. J., Soeldner, J. S., Jackson, R. & Eisenbarth, G. S. (1991) *Diabetes* **40,** 709–714.
32. Palmer, J. P., Asplin, C. M., Clemons, P., Lyon, K., Iatpati, O., Raghu, P. & Paquette, T. L. (1983) *Science* **222,** 1337–1339.
33. Keller, R. J. (1990) *J. Autoimmun.* **3,** 321–327.
34. Miller, A., Lider, O., Roberts, A. B., Sporn, M. B. & Weiner, H. L. (1991) *Proc. Natl. Acad. Sci. USA*, in press.
35. Miller, A. M., Lider, O. & Weiner, H. L. (1991) *J. Exp. Med.* **174,** 791–798.
36. Brod, S. A., Al-Sabbagh, A., Sobel, R. A., Hafler, D. A. & Weiner, H. L. (1991) *Ann. Neurol.* **29,** 615–622.
37. Khoury, S. J., Lider, O., Al-Sabbagh, A. & Weiner, H. L. (1990) *Cell. Immunol.* **131,** 302–310.