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Transgenic copper/zinc superoxide dismutase modulates susceptibility to type I diabetes

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ABSTRACT **A growing body of evidence suggests that active oxygen is an important participant in the destruction of the pancreatic β cell, which, in turn, leads to type I or insulin-dependent diabetes mellitus. Consequently, genetic factors predisposing susceptibility to insulin-dependent diabetes mellitus may include those that determine active oxygen metabolism. A direct test of this hypothesis is provided by a transgenic model for increased activity of Cu/Zn superoxide dismutase (EC 1.15.1.1), a principal radical scavenging enzyme. Here we demonstrate that elevated levels of this enzyme provided by a Cu/Zn superoxide dismutase transgene enhance the tolerance of pancreatic β cells to oxidative stress-induced diabetogenesis. These results show that this transgenic approach holds promise for revealing the role of reactive oxygen in autoimmune models of diabetogenesis as well as in other models of disease pathology in which active oxygen has been implicated.**

The pivotal cellular event in the etiology of type I or insulin-dependent diabetes mellitus (IDDM) is the loss of function and ultimately the complete disappearance of the insulin-producing pancreatic β cells. The development of IDDM is a complex multifactorial process, and environmental, dietary, viral, genetic, and autoimmune factors have all been implicated as contributing factors (1–4). A growing body of evidence suggests that active oxygen may be an important participant in the IDDM-related destruction of the β cell (5–7). For example, prediabetic immune infiltration of the pancreatic islet, a condition known as insulitis, is accompanied by release of cytotoxic reactive oxygen species including superoxide ($O_2^{\cdot-}$), hydroxyl radical (·OH), peroxynitrite ($ONOO^-$), and hypochlorite ($HOCl^-$). The diabetogenic drugs alloxan and streptozotocin also elicit β-cell destruction via reactive oxygen species (6, 8). Oxygen radical scavengers reduce the incidence of immune-mediated IDDM in the nonobese diabetic strain of mice and suppress the diabetogenic action of alloxan and streptozotocin (9–15). Collectively these observations suggest that reactive oxygen may be a common factor in several otherwise apparently disparate modes of diabetogenesis.

The pancreatic β cell appears to be exceptionally vulnerable to the cytotoxic action of reactive oxygen. Although the β cell appears to have somewhat reduced levels of some antioxidant enzymes (16–18), its total antioxidant status does not suggest this plays a significant role in diabetogenesis. Recent evidence suggests that the role of reactive oxygen in IDDM may best be understood in terms of another radical metabolite, nitric oxide (NO·). NO· is generated in β cells from L-arginine by constitutive and inducible isoforms of nitric oxide synthase (19) and appears to function in both negative and positive regulation of insulin secretion (20, 21). However, NO· reacts vigorously with superoxide to yield cytotoxic peroxynitrite (22, 23). Consequently, intrinsic NO· metabolism may render β cells vulnerable to levels of superoxide flux easily tolerated by other cell types. Further support for this concept comes from the observation that reduction of NO· levels by pharmacological inhibition of nitric oxide synthase activity significantly reduces β-cell cytotoxicity mediated either by cytokines (24, 25) or by alloxan and streptozotocin (26–28). Thus, the interaction of NO· and superoxide may be an important factor in the sensitivity of β cells to oxygen stress.

To further elucidate the role of oxygen radical metabolism in the genesis of IDDM, we asked whether genetic variation in oxygen radical metabolism can modulate susceptility to diabetogenesis. A transgenic mouse strain overexpressing the enzyme Cu/Zn superoxide dismutase (SOD, EC 1.15.1.1) provides an experimental system to simply and directly address this question. Cu/Zn SOD plays a pivotal role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide (H_2O_2), which, in mammals, is readily reduced to water principally by catalase or glutathione peroxidase. Unscavenged superoxide is an imminent threat to cells principally through transition metal-catalyzed reaction with H_2O_2 to yield the potent cytotoxic hydroxyl radical or, as mentioned, with NO· to yield peroxynitrite.

MATERIALS AND METHODS

Mice. Transgene heterozygotes and nontransgenic controls were generated by crossing TgHS-SF218/10 mice (29) with CD1. Segregating transgenic and nontransgenic animals were identified by PCR analysis or by SOD assay of tail biopsies (see below). Animals were maintained under specific pathogen-free conditions and fed standard mouse chow ad libitum.

SOD Assay. SOD activity in extracts was determined following separation of proteins in nondenaturing polyacrylamide gels after the method of Beauchamp and Fridovich (30). Tissues were sonicated in 2 mM EDTA/0.5% Nonidet P-40 (0.2 mg of tissue wet weight per ml).

Quantitative Assay of Transgene Transcripts by PCR. Quantitative multiplex reverse transcription-PCR was performed according to the method of Singer-Sam and coworkers (31) utilizing the ribosomal protein L32 (32) as a standard for relative expression levels. PCR reactions were performed with 0.2 μg of tissue-specific RNA isolated according to the method of Holtzman and coworkers (33). All PCR primers were between 21 and 24 bp in length and were designed to yield amplified cDNA fragments ranging in size between 300 and 500 bp. The SOD-MRT primer set amplifies a 452-bp

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Abbreviations: IDDM, insulin-dependent diabetes mellitus; SOD, superoxide dismutase.

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fragment from position 66 to position 518 of the mouse cDNA, the SOD-HRT primer set amplifies 430 bp from position 85 to position 515 of the human cDNA, and the SOD-BRT primer set amplifies 386 bp from position 16 to position 402 common to both cDNA sequences. The L32-specific primer set amplifies a 305-bp fragment from position 28 to position 333 of the respective cDNA. The final volume of the amplification cocktail was 25 μl and contained ≈20 pmol of each primer. The concentration of each primer was determined by titration (data not shown). The PCR profiles were one cycle at 50°C, 8 min, and 95°C, 2 min, for cDNA synthesis and 21–23 cycles at 90°C, 1 min, 60°C, 2 min, and 73°C, 2.5 min, for cDNA amplification. The reactions were then continued at 72°C for 10 min and cooled to 4°C. An aliquot (18 μl) of the amplification cocktail was loaded onto a 2.0% TBE gel containing 0.45 μg of ethidium bromide per ml and developed at 4 V/cm for 1 hr and 40 min. The image was photographed with Polaroid type 55 film and quantitation of the amount of PCR product was accomplished by densitometry with the aid of a Molecular Dynamics Densitometer, model 300E.

Drug Administration. After fasting for 8 hr, mice were anesthetized with methoxyflurane (Pitman–Moore, Washington Crossing, NJ) and injected via the tail vein with 50 mg of alloxan per kg of body weight (alloxan monohydrate in saline) or 0.2 ml of streptozotocin (24 mg/ml in 0.1 M citrate buffer). Control mice received 0.2 ml of saline or buffer as appropriate.

Blood Glucose Determination. Daily blood samples were analyzed for glucose using an ExacTech glucose monitor (MediSense, Cambridge, MA).

RESULTS AND DISCUSSION

The Cu/Zn SOD transgenic mouse strain, TgHS-SF218/10, carries a stable multiple copy insertion of a native human Cu/Zn SOD gene (29). Expression of this transgene supplements endogenous Cu/Zn SOD activity in a variety of tissues from 1.5 to 5 times normal levels. Extending this analysis, we now show that the human Cu/Zn SOD transgene is expressed in the transgenic pancreas as well. Quantitation of Cu/Zn SOD mRNA in the pancreas and, for comparison, the brain of TgHS-SF218/10 mice (Fig. 1; Table 1) demonstrates that the ratio of human to mouse mRNA in the pancreas is slightly

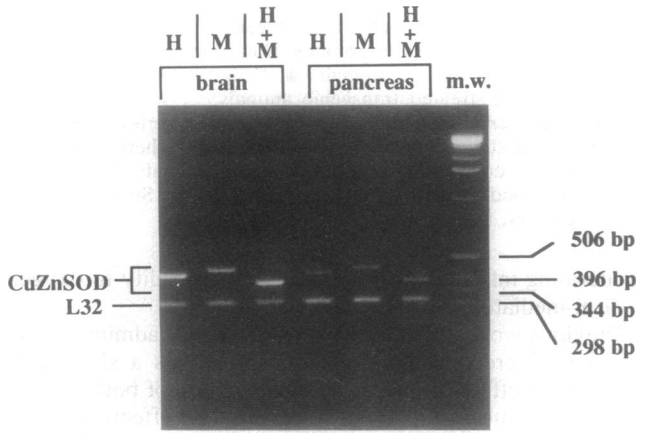

FIG. 1. PCR quantitation of human and mouse Cu/Zn SOD transcripts in the pancreas and brain of TgHS-SF218/10 transgenic mice. Primers were designed to amplify human (H), mouse (M), or human and mouse (H+M) transcripts and the transcript for an internal standard, ribosomal protein L32. The size of the amplified fragments is 430 bp for H transcripts, 452 bp for M transcripts, 386 bp for the H+M combined transcripts, and 305 bp for L32. m.w., Molecular weight standards.

Table 1. Cu/Zn SOD mRNA content of TgHS-SF218/10 mouse pancreas and brain

	Relative amount of Cu/Zn SOD mRNA*					
	Pancreas			Brain		
Experiment	H	M	H/M	H	M	H/M
1	0.165	0.118	1.40	2.75	1.25	2.20
2	0.295	0.325	0.91	1.97	1.17	1.68
Mean			1.16			1.94

H, human; M, mouse; H/M, human/mouse ratio.
*Determined by densitometric analysis relative to an internal standard (14).

greater than 1, compared to the brain, where the ratio is almost 2. Thus the level of total Cu/Zn SOD in the transgenic pancreas is expected to be about twice normal and in the transgenic brain about three times normal. Polyacrylamide gel assay of Cu/Zn SOD enzymatic activity in the pancreas and brain of transgenic animals (Fig. 2) shows the presence of the expected human homodimeric enzyme and human/mouse heterodimeric enzyme in these tissues. This confirms that the human transcripts are generating functional Cu/Zn SOD subunits that supplement total Cu/Zn SOD activity in the pancreas. Whether the transgene is expressed in the β cell of the pancreas is not known from these experiments. In any event, because the β cell represents only 1–2% of the total cell population of the pancreas, the proportion of β-cell-specific Cu/Zn SOD mRNA or enzyme activity in whole pancreatic extracts would be quite small.

To examine the capacity of transgenic Cu/Zn SOD to modulate susceptibility to oxygen radical-mediated diabetogenesis, TgHS-SF218/10 transgenic animals and their nontransgenic siblings, all 20–28 weeks of age, were initially challenged with the radical-generating diabetogenic drug, alloxan. Destruction of pancreatic β cells by alloxan is typically manifested by rapidly developing, permanent hyperglycemia (6, 8). Thus, hyperglycemia can be used as a reliable marker of β-cell cytotoxicity. Our nontransgenic control animals respond to alloxan with a steady rise in blood glucose for 5 days to a plateau level (≈15 mmol/liter) nearly three times above the steady-state blood glucose levels of sham-treated controls (≈5 mmol/liter) (Fig. 3). In contrast, the hyperglycemic response of TgHS-SF218/10 transgenic to alloxan is significantly suppressed. Blood glucose concentration in alloxan-treated transgenic animals rise to a plateau value of only 10 mmol/liter, an increase of about one-half of that of their nontransgenic siblings. Because the genetic backgrounds of the transgenic and nontransgenic control animals are identical, this effect can be attributed exclusively to the human Cu/Zn SOD transgene. Furthermore, all of our experiments utilized transgene heterozygotes to preclude any

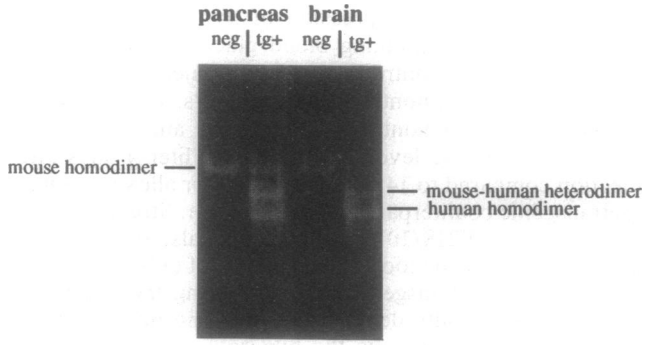

FIG. 2. Electrophoretic analysis of Cu/Zn SOD enzymatic activity in the pancreas and brain of TgHS-SF218/10 transgenic (tg+) and nontransgenic littermate control (neg) mice. The positions of the various dimeric forms of Cu/Zn SOD are indicated.

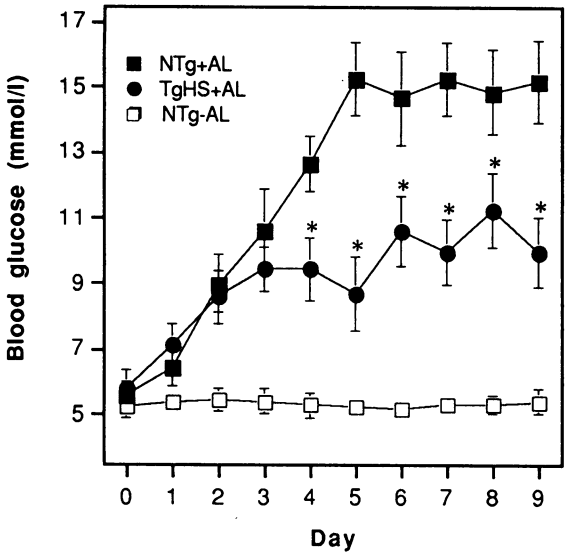

FIG. 3. Blood glucose levels in TgHS-SF218/10 heterozygotes (TgHS-SF218/10 × CD1) in response to alloxan administration. Each group of animals consisted of five males or females 20–28 weeks of age. Nontransgenic controls were segregating littermates. Data points are means ± SE of determinations on five animals. ■, Alloxan-treated nontransgenic animals; □, saline-treated nontransgenic animals; ●, alloxan-treated transgenic animals. Statistically significant blood glucose levels were determined by Student's *t* test. *, $P < 0.01$ vs. treated nontransgenic animals.

possible recessive effects on blood glucose levels arising from the chromosomal site of transgene insertion.

Whether the suppressed hyperglycemic response of TgHS-SF218/10 animals arises from transgene expression specifically in the pancreas or in other tissues cannot be determined from these experiments. To resolve this issue, we have generated mice with transgenic Cu/Zn SOD expression targeted exclusively to the β cell via the insulin promoter (H.M.K. and J.P.P., unpublished results). Regardless of the focus of transgenic Cu/Zn SOD activity, we believe that the increased resistance of TgHS-SF218/10 animals to alloxan-generated superoxide is clear evidence that genetic augmentation of Cu/Zn SOD can suppress the contribution of oxygen radicals to diabetogenesis.

To broaden the scope of our investigation, we examined the hyperglycemic response of younger (12–15 weeks) TgHS-SF218/10 animals to alloxan and to another diabetogenic drug, streptozotocin (Fig. 4). The diabetogenic effect of streptozotocin arises at least in part through a poorly understood radical-generating metabolism (see ref. 13 for review). As with their older counterparts, younger transgenic animals exhibit a significant suppression of hyperglycemia in response to alloxan, attaining blood glucose levels of ≈8.2 mmol/liter (59% of controls) 4 days after injection compared to 14 mmol/liter in nontransgenic controls. In response to streptozotocin, our nontransgenic control animals attained mean blood glucose levels of 26.4 mmol/liter 4 days after injection compared to 14 mmol/liter in their alloxan-treated, nontransgenic counterparts of the same age. Streptozotocin-treated TgHS-SF218/10 transgenic animals, on the other hand, attained mean blood glucose levels of only 17.1 mmol/liter (64% of nontransgenic siblings) during the same time period. These results demonstrate that the human Cu/Zn SOD transgene suppresses the hyperglycemic response to streptozotocin as well as to alloxan and that transgene expression is effective in younger as well as in older animals. These findings reinforce the general conclusion that genetic variation in Cu/Zn SOD expression can confer a broad

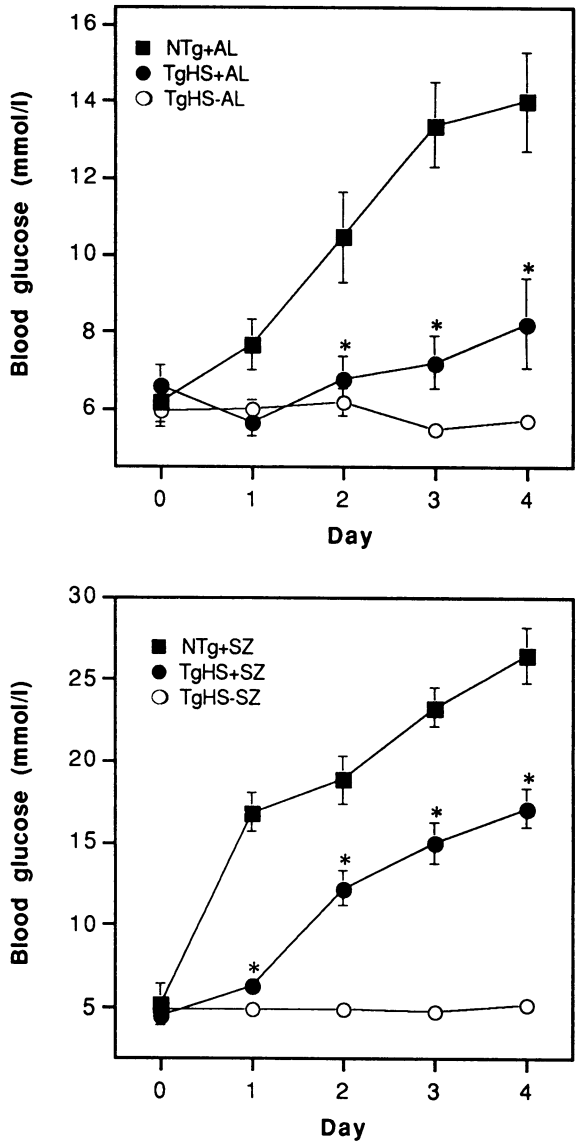

FIG. 4. Blood glucose levels in young TgHS-SF218/10 heterozygotes (TgHS-SF218/10 × CD1) in response to alloxan (*Upper*) or streptozotocin (*Lower*) administration. Each group of animals consisted of five males or females 12–15 weeks of age. Nontransgenic controls were segregating littermates. Data points are means ± SE of determinations on five animals. ■, Drug-treated nontransgenic animals; ●, drug-treated transgenic animals; ○, saline- or buffer-treated transgenic animals. Note that in this series, saline- or buffer-treated controls were transgenic animals, whereas in Fig. 3 saline-treated controls were nontransgenic animals. Statistically significant blood glucose levels were determined by Student's *t* test. *, $P < 0.01$ vs. treated nontransgenic animals.

moderating influence on individual susceptibility to oxygen radical-mediated diabetogenesis.

Previous work has shown that intravenous administration of purified preparations of Cu/Zn SOD has a short-term attenuating effect of the diabetogenic action of both alloxan and streptozotocin (10, 34). However, such effects are conferred by enzyme that almost certainly remains extracellular and that, therefore, may act by transiently augmenting the activity of the known extracellular species of SOD, EC-SOD (35). In contrast, endogenous Cu/Zn SOD appears to be exclusively intracellular (36, 37). Consequently, the attenuating effects of transgenic and injected Cu/Zn SOD on drug-induced hyperglycemia may arise through very different mechanisms.

Transgenic Cu/Zn SOD has been shown to confer broad protection against experimental acute oxidative damage in the central nervous system (38). This includes attenuation of focal cerebral ischemic injury (39), protection against cold-induced brain edema and infarction (40), and protection against drug-induced, superoxide-mediated neurotoxicity (41). The results presented in the present study extend the protective biological domain of transgenic Cu/Zn SOD to include the endocrine pancreas.

The rationale of the present experiments was to utilize the established radical-mediated diabetogenic properties of alloxan and streptozotocin as a short-term unambiguous assay for testing the capacity of Cu/Zn SOD transgenes to modulate oxygen radical-mediated diabetogenesis. Our findings confirm that both drugs elicit their effects through the generation of oxygen radicals and demonstrate that the diabetogenic action of these drugs can be attenuated by transgenic augmentation of Cu/Zn SOD. Thus, the answer to the question posed at the outset of whether genetic variation in oxygen radical metabolism can modulate susceptibility to diabetogenesis is clearly yes. This result sets the stage for asking whether genetic variation in oxygen radical metabolism can be a contributing factor in other diabetogenetic processes. As pointed out earlier, active oxygen may be an important participant in autoimmune or virally mediated diabetogenesis. Because we now know that transgenic augmentation of Cu/Zn SOD protects against oxygen radical-mediated diabetogenesis, we can now use our transgenic system to examine directly the role of active oxygen in these other diabetogenetic processes. Other laboratories have demonstrated that β-cell-targeted transgenic expression of the cytokines, α- and γ-interferon, or of the viral antigen, LCMV, elicits autoimmune insulitis leading to β-cell destruction (42–46). Generation of animals doubly transgenic for Cu/Zn SOD and autoimmune transgenes by simple Mendelian crosses can now provide the basis for an elegantly simple yet powerful system for elucidating the role of oxygen radical metabolism in these more biologically relevant diabetogenetic processes. A similar genetic approach could also be used to introduce Cu/Zn SOD transgenes into IDDM-susceptible nonobese diabetic mice (47–49). In principle, this approach can be used to investigate the role of reactive oxygen metabolism in any disease for which there is a mouse model.

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1. Dryberg, T. (1990) *Curr. Top. Microbiol. Immunol.* **156,** 1–137.
2. Eisenbarth, G. S. (1986) *N. Engl. J. Med.* **314,** 1360–1368.
3. Gepts, W. & LeCompte, P. M. (1985) in *The Diabetic Pancreas*, eds. Volk, B. W. & Arguilla, B. R. (Plenum, New York), pp. 337–365.
4. Leiter, E. H. (1989) *FASEB J.* **3,** 2231–2241.
5. Lowenstein, C. J. & Snyder, S. H. (1992) *Cell* **70,** 705–707.
6. Oberley, L. W. (1988) *Free Radical Biol. Med.* **5,** 113–124.
7. Ward, P. A., Warren, J. S. & Johnson, K. J. (1988) *Free Radical Biol. Med.* **5,** 403–408.
8. Rerup, C. C. (1970) *Pharmacol. Rev.* **22,** 485–518.
9. Fischer, L. J. & Hamberger, S. A. (1980) *Diabetes* **29,** 213–216.
10. Gandy, S. E., Buse, M. G. & Crouch, R. K. (1982) *J. Clin. Invest.* **70,** 650–658.
11. Nomikos, I. N., Wang, Y. & Laferty, K. J. (1989) *Immunol. Cell Biol.* **67,** 85–87.
12. Robbins, M. J., Sharp, R. A., Slonim, A. E. & Burr, I. M. (1980) *Diabetologia* **18,** 55–58.
13. Sandler, S., Welsh, M. & Andersson, A. (1983) *Acta Pharmacol. Toxicol.* **53,** 392–400.
14. Sumoski, W., Baquerizo, H. & Rabinovich, A. (1989) *Diabetologia* **32,** 792–796.
15. Yamada, K., Nonaka, K., Hanafusa, T., Miyazaki, A., Toyoshima, H. & Tarui, S. (1982) *Diabetes* **31,** 749–753.
16. Gandy, S. E., Galbraith, R. A., Crouch, R. K., Buse, M. G. & Galbraith, G. M. (1981) *N. Engl. J. Med.* **204,** 1547–1548.
17. Grankvist, K., Marklund, S. & Taljedal, I. (1981) *Biochem. J.* **199,** 393–398.
18. Malaisse, W. J., Malaisse-Lagae, F., Sener, A. & Pipeleers, D. G. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 927–930.
19. Corbett, J. A., Sweetland, M. A., Wang, J. L., Lancaster, J. R. J. & McDaniel, M. L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1731–1735.
20. Schmidt, H. H. H. W., Warner, T. D., Ishii, K., Seng, H. & Murad, F. (1992) *Science* **225,** 721–723.
21. Corbett, J. A., Mikhael, A., Shimizu, J., Frederick, K., Misko, T. P., McDaniel, M. L., Kanagawa, O. & Unanue, E. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8992–8995.
22. Beckman, J. S. (1991) *J. Dev. Physiol.* **15,** 53–59.
23. Ischiropoulos, H., Zhu, L. & Beckman, J. S. (1992) *Arch. Biochem. Biophys.* **298,** 446–451.
24. Corbett, J. A. & McDaniel, M. L. (1992) *Diabetes* **41,** 897–903.
25. Corbett, J. A., Tilton, R. G., Chang, K., Hasan, K. S., Ido, Y., Wang, J. L., Sweetland, M. A., Lancaster, J. R., Jr., Williamson, J. R. & McDaniel, M. L. (1992) *Diabetes* **41,** 552–556.
26. Kolb, H., Kiesel, U., Kroncke, K.-D. & Kolb-Bachofen, V. (1991) *Life Sci.* **49,** 213–217.
27. Kolb, H. & Kolbbachofen, V. (1992) *Diabetologia* **35,** 796–797.
28. Lukic, M. L., Stosic-Grujicic, S., Ostojic, N., Chan, W. L. & Liew, F. Y. (1991) *Biochem. Biophys. Res. Commun.* **178,** 913–920.
29. Epstein, C. J., Avraham, K., Lovett, M., Smith, S., Elroy-Stein, O., Rotman, G., Bry, C. & Groner, Y. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8044–8048.
30. Beauchamp, C. O. & Fridovich, I. (1971) *Anal. Biochem.* **44,** 276–287.
31. Singer-Sam, J., Robinson, M. O., Belle, A. R., Simon, M. I. & Riggs, A. D. (1990) *Nucleic Acids Res.* **18,** 1255–1259.
32. Dudov, K. P. & Perry, R. P. (1984) *Cell* **37,** 457–468.
33. Holtzman, D. M., Bayney, R. M., Li, Y. W., Khosrovi, H., Berger, C. N., Epstein, C. J. & Mobley, W. C. (1992) *EMBO J.* **11,** 619–627.
34. Grankvist, K., Marklund, S. & Taljedal, I. (1981) *Nature (London)* **294,** 158–160.
35. Hjalmarsson, K., Marklund, S., Engstrom, A. & Edlund, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6340–6344.
36. Keller, G.-A., Warner, T. G., Steimer, K. S. & Hallewell, R. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7381–7385.
37. Crapo, J. D., Oury, T., Rabouille, C., Slot, J. W. & Chang, L. Y. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10405–10409.
38. Epstein, P. N., Boschero, A. C., Atwater, I., Cai, X. G. & Overbeek, P. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12038–12042.
39. Kinouchi, H., Epstein, C. J., Mizui, T., Carlson, E., Chen, S. F. & Chan, P. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11158–11162.
40. Chan, P. H., Yang, G. Y., Chen, S. F., Carlson, E. & Epstein, C. J. (1991) *Ann. Neurol.* **29,** 482–486.
41. Przedborski, S., Kostic, V., Jacksonlewis, V., Naini, A. B., Simonetti, S., Fahn, S., Carlson, E., Epstein, C. J. & Cadet, J. L. (1992) *J. Neurosci.* **12,** 1658–1667.
42. Ohashi, P. S., Oehen, S., Buerki, K., Pircher, H., Ohasni, C. T., Odermatt, B., Malissen, B., Zinkernagel, R. M. & Hengartner, H. (1991) *Cell* **65,** 305–317.
43. Oldstone, M. B. A., Nerenberg, M., Southern, P., Price, J. & Lewicki, H. (1991) *Cell* **65,** 319–331.
44. Sarvetnick, N., Liggitt, D., Pitts, S. L., Hansen, S. E. & Stewart, T. A. (1988) *Cell* **52,** 773–782.
45. Sarvetnick, N., Shizuru, J., Liggett, D., Martin, L., McIntyre, B., Gregory, A., Parslow, T. & Stewart, T. (1990) *Nature (London)* **346,** 844–847.
46. Stewart, T. A., Hultgren, B., Huang, X., Pitts-Meek, S., Hully, J. & MacLachlan, N. J. (1993) *Science* **260,** 1942–1946.
47. Castano, L. & Eisenbarth, G. S. (1990) *Annu. Rev. Immunol.* **8,** 647–679.
48. Hanafusa, T. & Tarui, S. (1990) *Curr. Top. Microbiol. Immunol.* **156,** 15–25.
49. Katz, J. D., Wang, B., Haskins, K., Benoist, C. & Mathis, D. (1993) *Cell* **74,** 1089–1100.