THE LANCET

Peptide therapy for diabetes in NOD mice

Dana Elias Irun R Cohen

Reprinted from THE LANCET Saturday 19 March 1994
Vol. 343 No. 8899 Pages 704–706

THE LANCET 42 BEDFORD SQUARE LONDON WC1B 3SL UK

Peptide therapy for diabetes in NOD mice

Dana Elias, Irun R Cohen

NOD mice spontaneously develop autoimmune diabetes that mimics insulin-dependent diabetes mellitus (IDDM) in man. A peptide of the 60 kDa heat shock protein (hsp60), designated p277, can serve as a target for diabetogenic T-cell clones, and diabetes was prevented by using the p277 peptide to turn off anti-p277 immunity early in life. We report that the p277 peptide, administered once, can arrest the autoimmune process even after it is far advanced. Successful therapy was associated with down-regulation of the autoimmune process and regression of islet inflammation. Thus the immune system is responsive to manipulation by a specific signal even in the face of a virulent, full-blown autoimmune process.

Lancet 1994; **343**: 704–06

See Commentary page 684

The collapse of glucose homoeostasis and clinical insulin-dependent diabetes mellitus (IDDM, type 1 diabetes) is thought to occur only after 80–90% of pancreatic β cells have been inactivated by the immune system. Incipient pre-clinical diabetes can be diagnosed by the detection of immunological markers of β-cell autoimmunity only after the onset of the autoimmune process. Can the immune system be turned back once it has started on the course to disaster? We have examined this question by studying spontaneous diabetes in NOD mice, which is considered to be a good model of human IDDM.[1,2] Female NOD mice develop insulitis around 4 weeks of age. Hyperglycaemia begins at about 14–17 weeks. By 35–40 weeks almost all female NOD mice have developed severe diabetes and most die in the absence of insulin treatment.

The initiation of the autoimmune process in NOD mice can be prevented by, for instance, restricted diet, viral infections, or non-specific stimulation of the immune system.[1] NOD diabetes is also preventable by induction of immunological tolerance in pre-diabetic mice to the antigen glutamic acid decarboxylase.[3] We have found that NOD diabetes can be prevented by vaccination with T cells specific for the p227 peptide fragment of the heat shock protein 60 kDa (hsp60) molecule.[4] Administration of the p277 peptide itself at the onset of insulitis also prevented the development of diabetes, probably by down-regulating the anti-p277 immunity that is essential for NOD diabetes.[4] But prevention is not treatment. The present experiments were done to test whether the p277 peptide could be used to regulate established β-cell autoimmunity.

We administered 50 μg of the p277 peptide to female NOD mice just before or after (12, 15, and 17 weeks of age) the appearance of overt hyperglycaemia. The control treatment was 50 μg bovine serum albumin (BSA, Sigma) in oil, another antigen implicated in IDDM.[5] The antigens were diluted in normal saline and emulsified in an equal volume of mineral oil (incomplete Freund's adjuvant, Difco). Mice were treated with the emulsions of p277 or of BSA in volumes of 0·1 mL injected subcutaneously in the back. Blood glucose was measured at 1000 h in non-fasting mice[4] at the time of treatment and, in those that survived, at age 40 weeks. Significant hyperglycaemia was taken as a glucose concentration of 11·1 μmol/L or greater, because this concentration was three standard deviations higher than the mean in 100 healthy mice.

At the time of treatment, all 20 of the 12-week-old mice had normal blood glucose, and about half of the 21 15-week-old mice, and 16 of the 20 17-week-old mice were hyperglycaemic (figure). Of the total of 30 mice in the three groups that received BSA, 28 were dead of severe diabetes by 40 weeks. By contrast, none of the 21 mice treated with p277 at 12 or 15 weeks of age died, and only 1 of the 10 treated with p277 at 17 weeks died, despite the absence of insulin treatment. Most of the mice manifested mild hyperglycaemia and a few were euglycaemic and apparently cured of diabetes. Some hyperglycaemic mice became euglycaemic after p277 treatment. Similar results were obtained in other experiments in which a total of 157 NOD mice were treated with p277 and 100 were treated with BSA at 7, 12, 15, or 17 weeks of age. The overall mortality by 40 weeks in the BSA-treated mice was 84%. The mortality in the p277-treated mice was only about 3%, and all of the dead mice were among those already severely diabetic at treatment.

Arrest of the disease process was associated with regression of insulitis. For example, histological examination of the pancreas at age 23 weeks in mice that had been treated at 12 weeks showed a difference between the p277-treated and the BSA-treated mice: 36% of islets in p277-treated mice but 0% of those in BSA-treated mice were free of insulitis.

hsp60 is expressed with insulin in the secretory granules of β cells.[6] Perhaps this expression of hsp60, apparently unique to β cells, is the reason why anti-p277 autoimmune T cells attack β cells. However, if anti-p277 T cells damage β cells, how does administration of p277 arrest the diabetogenic process? We have found in other experiments that administration of p277 leads to activation of T cells

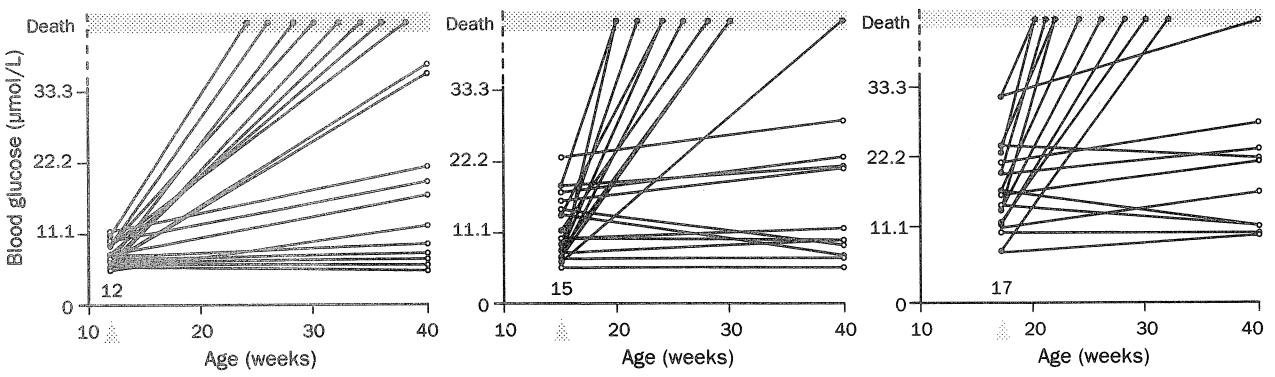

Figure: **Treatment (arrow) of NOD mice with p277 (○) or BSA (●)**

specific for a T-cell receptor characteristic of anti-p277 T cells. Thus administration of p277 activates a network of anti-idiotypic T cells. Such anti-idiotypic T cells appear actively to down-regulate or suppress the effector T cells that destroy β cells. Anti-idiotypic T cells do down-regulate autoimmune T cells in animal diseases[7] and clinically in multiple sclerosis.[8] How p277 induces anti-idiotypic regulatory T cells remains to be clarified. Nevertheless, the beneficial effects of p277 on the disease are clear. We conclude that the behaviour of the mouse immune system is susceptible to specific modification by the information contained in the p277 peptide. Other autoimmune diseases may also be treatable with peptides specific for the particular disease.

In view of the similarity between the diabetes of NOD mice and IDDM in man, we have begun to test newly diagnosed IDDM patients for T-cell reactivity to peptide p277. We found positive responses in 19 of the first 23 patients; healthy controls showed no reactivity to p277. Thus treatment with p277 peptide may be beneficial in the human diabetogenic process. The results with p277 in NOD mice support the idea that the immune system is a cognitive system in which behaviour can be self-correcting provided the system is given suitable information.[9,10]

DE was supported by a postdoctoral fellowship granted by the Juvenile Diabetes Foundation International. The work was supported by grants from the Minerva Foundation, the Tauro Foundation, and Mr Rowland Schaeffer.

References

1 Castano L, Elsenbarth GS. Type-1 diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu Rev Immunol* 1990; **8**: 647–79.

2 Bendelac A, Carnaud C, Boitard C, Bach JF. Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates: requirement for both L3T4+ and Ly+2+T cells. *J Exp Med* 1987; **166**: 823–32.

3 Tisch R, Yang XD, Singer SM, Liblav RS, Fuggar L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature* 1993; **366**: 72–75.

4 Elias D, Reshef T, Birk OS, van der Zee R, Walker MD, Cohen IR. Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65 kDa heat shock protein. *Proc Natl Acad Sci USA* 1991; **88**: 3088–91.

5 Karjalainen J, Martin JM, Knip M, et al. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med* 1992; **327**: 302–07.

6 Brudzynski K, Martinez V, Gupta RS. Immunocytochemical localization of heat-shock protein 60-related protein in beta-cell secretory granules and its altered distribution in non-obese diabetic mice. *Diabetologia* 1992; **35**: 316–24.

7 Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR. Anti-idiotypic network induced by T cell vaccination against EAE. *Science* 1989; **239**: 181–83.

8 Zhang J, Medaer R, Stinissen P, Hafler D, Raus J. MHC-restricted depletion of human myelin basic protein-reactive T cells by T cell vaccination. *Science* 1993; **261**: 1451–54.

9 Cohen IR. The cognitive principle challenges clonal selection. *Immunol Today* 1992; **13**: 441–44.

10 Cohen IR. The cognitive paradigm and the immunological homunculus. *Immunol Today* 1992; **13**: 490–94.

Department of Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel (D Elias PhD, Prof I R Cohen MD)

Correspondence to: Prof Irun R Cohen

Printed in UK
by Robquest Ltd