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Transgenic overproduction of murine 60 kDa heat shock protein in the liver does not prevent type I diabetes in NOD mice

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To the Editor:

Type I diabetes is an autoimmune disease in which insulin-producing beta cells of the pancreas are selectively destroyed. Multiple islet cell molecules have been suggested as the target of autoimmunity in man and in the mouse model of the non-obese diabetic (NOD) mouse, including (pro-) insulin, GAD and heat shock protein 60 (HSP 60) [1]. Of these, HSP 60, its immunodominant peptide (p277) in particular, has been shown to protect NOD mice against diabetes in NOD mice [2], and the results of a Phase II trial indicate that treatment of patients with p277 can preserve endogenous insulin production [3].

To address the potential role of HSP 60 as an autoantigen in diabetes, the murine *Hspdl* gene has been overexpressed in the thymus of NOD mice [4]. Although this did not result in thymic deletion of HSP 60-reactive T cells or abrogation of diabetes, diabetes susceptibility was diminished. Thus, the resistance to diabetes in these mice seemed to involve non-deletional tolerance mechanisms. However, other groups have reported that p277 immunisation does not affect diabetes in mice [5], and that intrathymic administration of HSP65 or p277 may even accelerate the onset of diabetes [6].

To determine whether overproduction of self HSP 60 in a peripheral organ could also influence diabetes susceptibility or T-cell autoimmunity, we generated transgenic mice that overexpress the murine *Hspdl* gene in liver; the liver was chosen for its known tolerogenic properties [7]. To that end, the murine *Hspdl* cDNA ligated to the promoter of the gene for human C-reactive protein was microinjected into fertilised FVB oocytes, as described previously [8], and two founders were established, the offspring of which

showed 5- to 10-fold higher HSP 60 production in liver compared with non-transgenic littermates, as tested by western blotting. Transgenic offspring of both founder lines (R1 and R3) were then backcrossed over ten generations onto the diabetes-susceptible NOD/LtJ background to obtain NOD congenic *Hspdl* transgenic mice. Mice were bred and kept in the specific pathogen-free animal facility at the Johannes Gutenberg-University (Mainz, Germany), and considered diabetic at blood glucose levels above 11 mmol/l (assessed with Accucheck blood glucose strips, Roche, Mannheim, Germany).

Hspdl transgenic NOD mice of both the R1 and R3 line showed a similar onset and rate of disease progression to diabetes compared with non-transgenic NOD mice (Fig. 1). At 24 weeks and 40 weeks of age the incidence of diabetes in *Hspdl* transgenic NOD R1 mice ($n=13$) was 46 and 69%, compared with 56 and 67% in non-transgenic littermates ($n=9$), respectively; the difference between these groups, as assessed by Fisher's exact test, was not significant. Splenocytes obtained from *Hspdl* transgenic NOD mice or non-transgenic littermates at week 12 showed a similar spontaneous proliferative response to stimulation with recombinant murine HSP 60 protein (Stimulation index 10.2 vs 9.2) or synthetic p277 peptide (Stimulation index 8.5 vs 8.9), at a dose of 10 µg/ml protein or peptide. These findings show that *Hspdl* overexpression in the liver does not eliminate T-cell responses to self HSP 60.

In conclusion, *Hspdl* overexpression in a tolerogenic peripheral organ was not sufficient to diminish diabetes susceptibility in NOD mice, in contrast to *Hspdl* overexpression in the thymus [4]. However, neither thymic nor peripheral overexpression of *Hspdl* could entirely prevent diabetes. Thymic or hepatic overexpression of *Hspdl* did not eliminate or alter T-cell responses to self HSP 60 or p277. Therefore, it is possible that the T-cell response to HSP 60 antigen is not the critical determinant of disease susceptibility in NOD mice but, rather, a modulator of diabetogenic immunity, since self HSP 60 has been found to modulate immune responses by binding to innate receptors of the toll-like receptor family [9]. Thus, HSP

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Fig. 1 Diabetes in female *Hspdl* transgenic NOD R1 mice ($n=13$; dashed lines) or non-transgenic mice ($n=9$; continuous lines). Shown are the longitudinal blood glucose values (mean and SEM; lines with symbols) and diabetes incidence (%; lines without symbols)

60 signalling to antigen-presenting cells or T cells may result in a non-diabetogenic T-cell phenotype.

Duality of interest The authors are not aware of any conflicts of interest.

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