Response from the authors

Dear Sir,
We thank Dr. Torffvit for his query regarding the efficacy of different forms of insulin in the rat. Our own study has not specifically addressed this issue. However, over the last 10 years, we have used different doses of both bovine and human ultralente insulins in rats with both short- and long-term experimental diabetes. We were unable to locate a direct comparison of bovine and human insulin in rats although this issue has been addressed in a range of human studies. These studies have suggested that human ultralente insulin, although similar in potency to bovine insulin, has a shorter duration of action in man [1, 2].

In our previous animal studies, we used heat-treated bovine Ultralente insulin and as noted by Dr. Torffvit, we achieved blood glucose levels of less than 22 mmol/l with a dose of 2 IU/day [3]. However, as indicated previously, there was a heterogeneous response among the diabetic rats. In our more recent studies, using human ultralente insulin, the dose of 2 IU/day was not associated with blood glucose levels greater than 20 mmol/l [4]. However, the possibility that human insulin is ineffective in rats is unlikely since in a recent study using human insulin administered via silastic implants, we were able to achieve euglycaemia [5].

Another factor which needs to be considered relates to the severity of the diabetes that is induced by streptozotocin. This relates not only to the susceptibility of the individual rat strain to streptozotocin but also to the dose and mode of administration of streptozotocin [6]. In our studies, streptozotocin was given at a dose of about 45–60 mg/kg whereas Dr. Torffvit gave a much higher dose of 90 mg/kg. Our own experience involving serial sampling of diabetic animals for blood glucose levels has indicated marked temporal variability of glucose control within individual animals. This emphasises the importance of using glycated haemoglobin in long-term studies in diabetic rats. Finally, it is possible to obtain both bovine insulin (Ultralente MC beef) and human insulin (Ultratard Human Monocomponent) from Novo Nordisk (Copenhagen, Denmark).

Yours sincerely,
T. Soulis-Liparota, M. Cooper, M. Dunlop, G. Jerums

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Class I HLA is associated with age-at-onset of IDDM, while class II HLA confers susceptibility to IDDM

Dear Sir,
Among patients with insulin-dependent diabetes mellitus (IDDM), heterogeneity is observed for age of the disease onset. Previous reports in different ethnic groups suggested that the heterogeneity in age-at-onset of IDDM may be determined by gene(s) in the HLA region [1–5]. To investigate the relative contribution of HLA loci to genetic susceptibility to IDDM and to heterogeneity in IDDM, we typed 10 loci in the HLA region, i.e. HLA-A, B, C, DR, DQA1, DQB1, TAP1, TAP2, LMP2 and HSP70-2 genes.

Eighty-one unrelated patients with IDDM and 71 healthy control subjects were studied. Mean ± SD age-at-onset of the disease was 20.8 ± 13.8 years (range: 0–59 years). The patients were divided into tertiles according to their age-at-onset of IDDM, such that each group contained an equal number of patients: early-onset (0–12 years), intermediate-onset (13–24 years), and late-onset (> 25 years). HLA-A, HLA-B, HLA-C and HLA-DR antigens were serologically typed. HLA-DQA1 and DQB1 alleles and polymorphism of TAP1, TAP2 and LMP2 genes were determined by PCR-RFLP methods as reported previously [1, 6, 7]. A Pst I polymorphism of heat shock protein (HSP) 70–2 gene was examined by the Southern blot hybridization method with probe (American Type Culture Collection, Rockville, Md., USA) [8]. The significance of the difference in allele frequencies was determined by the chi-square test or Fisher's exact test, with corrected p values (pc).

As reported previously [1], susceptibility to IDDM was strongly associated with class II HLA loci. HLA-DR9, DQA1*0301, DQB1*0303 and DQB1*0401 were positively associated with IDDM, whereas HLA-DR2, DQA1*01, DQA1*0103, DQB1*0501 and DQB1*0601 were negatively associated. In contrast to the strong association of class II alleles with disease susceptibility, class II alleles showed little effect on the age of disease onset. Instead, class I loci were strongly associated with the age-at-onset of IDDM. Significant heterogeneity was observed in the distribution of B7 and Cw7 alleles. The frequency of B7 was highest in early-onset patients (35 %), was 4.4 % in the intermediate age-at-onset group, and none of the late-onset patients had this allele ($\chi^2 = 15.0$, 2 degrees of freedom [df], uncorrected $p = 0.0006$,

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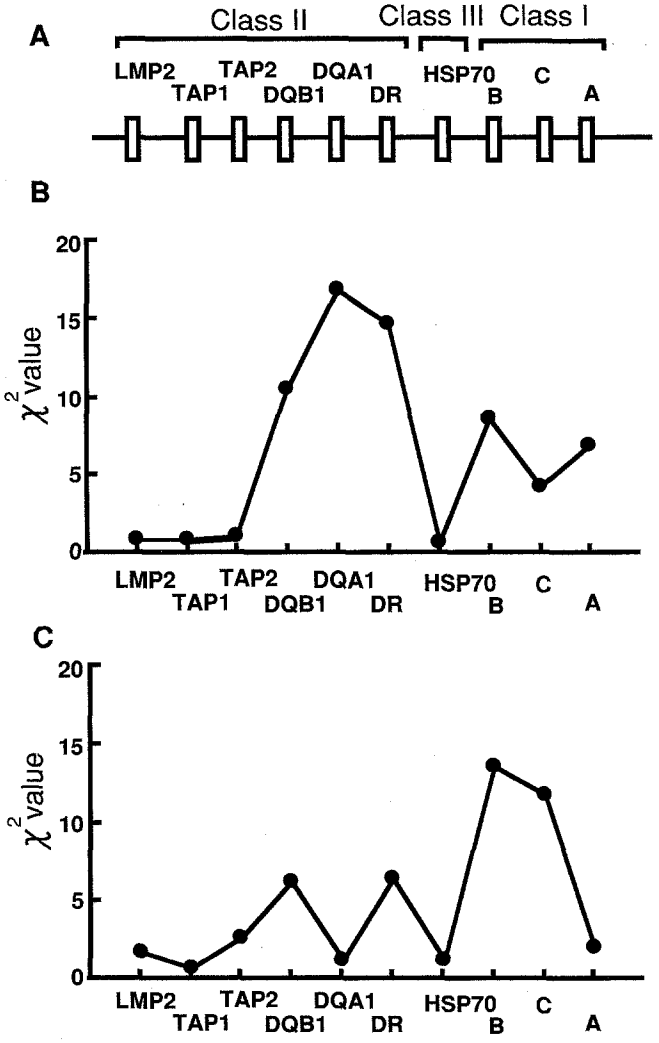

Fig. 1. **A:** Genetic map of HLA. **B:** Chi-square value associated with each allele giving the maximum chi-square values for 2×2 contingency table (1 *df*) at each locus for IDDM (vs control subjects). The alleles were: LMP2-60: His(+), TAP1-333: Val(+), TAP2-687: stop(−), DQB1: *0601(−), DQA1: *0301(+), DR: 2(−), HSP70: 8.5 kb(−), B: 54(+), C: w1(+), A: 31(−). **C:** chi-square value associated with each allele giving the maximum chi-square values for 2×3 contingency table (2 *df*) at each locus for age-at-onset of IDDM. The alleles were: LMP2–60: His(−), TAP1–333: Val(+), TAP2–687: stop(−), DQB1: *0501(+), DQA1: *0301(+), DR: 1(+), HSP70: 8.5 Kb(+), B: 7(+), C: w7(+), A: 24(+)

$pc = 0.02$). The frequency of the B7 allele in the early-onset group was significantly higher than that in the intermediate plus late-onset group (relative risk (RR): 25.3, 95% confidence interval (CI): 4.8–133.4, $pc = 0.01$). The mean ± SEM age-at-onset of the patients with HLA-B7 was significantly lower than that in patients without this antigen (9.5 ± 1.9 vs 24.1 ± 1.8 years, $p < 0.002$, Mann-Whitney *U*-test). Significant heterogeneity in the distribution was also observed for HLA-Cw7 ($\chi^2 = 12.6$, 2 *df*, uncorrected $p = 0.002$, $pc = 0.01$). A weak, but not significant, heterogeneity was observed for HLA-DR1 and DQB1*0501 ($\chi^2 = 7.0$ and 5.4, respectively, 2 *df*, $pc > 0.05$). TAP1, TAP2, LMP2 and HSP70 loci showed little effect on age-at-onset of IDDM.

Simultaneous typing of 10 loci in the same set of subjects in this study enabled us to assess the relative contribution of each locus to susceptibility to IDDM and to heterogeneity in the age-at-onset of IDDM. As shown in Figure 1, class II loci, DQ and DR loci in particular, were associated with susceptibility to IDDM, whereas class I loci, the B and/or C loci in particular, were strongly associated with age-at-onset of IDDM.

Although B7 and Cw7 were strongly associated with age-at-onset of IDDM, this does not necessarily indicate that class I B or C locus itself affects the age-at-onset of IDDM. There are many genes close to the class I genes that are being characterised at the present time and any one of them that is in linkage disequilibrium with the B7 and/or Cw7 alleles could be responsible for this effect. However, our data suggest that a gene (or genes) responsible for this effect map(s) to the class I, but not class II, region of the HLA complex. Following submission of this manuscript, Demaine et al. [9] reported an association of a new marker, P3B, located in the HLA class I region, with age at onset of IDDM, in the UK. They showed linkage disequilibrium between the new marker and HLA-A, B, but they did not type HLA-C. These data suggest that age-at-onset of IDDM is determined by a gene (or genes) which is in linkage disequilibrium with class I B, C or P3B locus.

Class I and class II HLA molecules play an important role in immune response. Since HLA class II molecules play a role in triggering this response, it is reasonable to speculate that class II HLA affects the initial pathogenic events in the development of IDDM. On the other hand, HLA class I molecules play a role in the final step of the immune response, resulting in the destruction of target cells. Thus, class I HLA loci may affect the rate or degree of pancreatic beta-cell destruction, and thereby may be associated with the age-at-onset of IDDM as observed in this study. Several studies in both humans and in animal models suggest the contribution of class I MHC loci to the pathogenesis of IDDM [10–12].

In conclusion, our data suggest that class I HLA is associated with age-at-onset of IDDM. These findings emphasize the importance of class I HLA, in addition to class II HLA, in the pathogenesis of IDDM.

Yours sincerely,
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Insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene

Dear Sir,
We read with great interest the recent paper published in Diabetologia by Tarnow et al. [1].

A considerable part of the data in this paper is also contained in a paper recently published in Diabetes [2] by the same group. These areas include the 'Subjects and methods' section which is very similar between the two papers. The data in Table 1 of both papers is more than 75 % identical.

The beginning of the 'Results' section contains identical data and the text is the same. Both papers present the results of angiotensin converting enzyme (ACE) genotyping in patients with nephropathy compared to those with normoalbuminuria. The Diabetes paper also presents these data in a Table. Both papers have presented the same plasma ACE levels with respect to ACE genotypes as well as nephropathy and normoalbuminuria. The Diabetes paper has also presented the data in a Table.

It is of particular interest that both papers were received on the same day (10 October 1994) in the editorial offices of Diabetologia and Diabetes. The Diabetologia paper makes only a brief reference to the Diabetes paper [ref. 36]. The revised form of the Diabetologia paper was received on 20 December 1994 whilst the revised Diabetes paper was received on 19 January 1995. With respect to the Diabetes paper the authors must have known that the Diabetologia paper was likely to be accepted yet no mention of its existence or the data regarding coronary heart disease (CHD) has been made. Consequently, the discussion in the Diabetes paper has been written with no mention of the role of CHD in nephropathy despite the authors' having a paper which looks at CHD in exactly the same group of patients. This is very misleading for the reader and the discussion in the Diabetes paper has been written without disclosing the full facts.

Finally, the 'Statement to be signed by all authors' for submission of manuscripts to Diabetologia states 'We confirm that neither the manuscript submitted nor any part of it has been published or is being considered for publication elsewhere . . . ' clearly this is not the case. Likewise, Diabetes also requires a statement where 'authors must state in their transmittal letter that the material has not been published or submitted simultaneously to another journal'.

We believe that it is very poor scientific practice for a group to attempt to publish data twice.

Yours sincerely,
A. Demaine, M. Hibberd, A. Millward

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Response from the authors

Dear Sir,
Drs. A. Demaine, M. Hibberd, and A. Millward believe 'that it is very poor scientific practice for a group to attempt to publish data twice'. We completely agree and consequently we have never done so and do not intend to in the future. Actually, Diabetologia received a full copy of the paper submitted to Diabetes before acceptance. However, we are puzzled and astonished over the accusation since we are planning to publish many more papers (an additional one has already been accepted by Diabetes and another two have been submitted to Diabetes/Diabetologia) based on our large case/control study consisting of 198 insulin-dependent diabetic (IDDM) patients with diabetic nephropathy and a matched group of 190 IDDM patients with normoalbuminuria. The two groups have been carefully evaluated in relation to demographic data, microangiopathy, macroangiopathy, autonomic neuropathy, cardiovascular risk factors, endothelial dysfunction etc. DNA was isolated from all patients. The reason for conducting such a large case/control study was to answer questions within the following topics:
1. Importance of genetic abnormalities in relation to initiation and progression of diabetic nephropathy and diabetic retinopathy.