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Letters

Tolerance loss in diabetics: association with foreign antigen exposure

We read with interest the article by Dr Lawlor's group relating milk avoidance to insulin resistance [1]. Cow's milk has also been causally associated with Type 1 diabetes [2]. However, the pathogenic basis of these associations remains unclear. We hypothesize that the possible role of cow dairy in causing/accentuating the diabetic state could be due to a loss of oral tolerance leading to a burden on the individual's immune defence systems. Data in support of this hypothesis are presented below.

Employing an advanced *ex vivo* lymphocyte response assay (LRA by ELISA/ACT) we identify patient-specific immunoreactivity to over 400 antigens in various clinical settings [3]. Recently, in a community-based randomized clinical trial, we identified such reactivities in cohorts of patients with Type 1 (n = 27) and Type 2 (n = 26) diabetes. Patients undertook conventional 'best practices' diabetes management alone (control arm, 13 in each cohort) or additionally carried out a novel patient-specific comprehensive care protocol (test arm, 14 Type 1 and 13 Type 2 diabetics): test patients substituted for their reactive antigens and were advised to follow a repair-stimulating diet including nutrient supplementation plans as detailed previously [4]. Biweekly support group meetings were held where qualified nutritionists provided on-going guidance and instruction to implement their plan. Control subjects attended separate support groups in which the nutritionists provided guidance consistent with American Diabetes Association guidelines. Long-term glycemic control was assessed by changes in HbA_{1c} levels over a 6-month study period.

Individual immune reactants were highly variable. More reactivity was seen in Type 1 than in Type 2 diabetics (Fig. 1). The single most common immune reactant was cow dairy (69% of Type 2 and 28% of Type 1 diabetics; $P < 0.01$). Although reactivity to environmental chemicals and additives was greater, there was no predilection for any single antigen within these groups. This finding is more striking because asymptomatic (healthy) people show no hypersensitivities (they are tolerant and with functional homeostatic mechanisms; unpublished data).

Glycemic control improved following the 6-month comprehensive care protocol. The fall in average HbA_{1c} levels was significant among Type 2 diabetics (13.3% reduction in test vs. 2.6% in control subjects; $P < 0.05$). Although values for Type 1 diabetics did not attain statistical significance, the decrease in HbA_{1c} levels was greater in test (8.7% reduction) compared with control subjects (5.2%). Importantly, six of 14 (42.86%) Type 1 diabetes test subjects had a reduction in HbA_{1c} levels of ≥ 1.0, while only three of 11 (27.3%) control subjects had this degree of reduction ($P < 0.05$). All but three Type 1 diabetes test subjects achieved an HbA_{1c} level of ≤ 7, thus achieving the ideal situation of glycemic control. In Type 2 diabetes, values remained above 7, suggesting that additional time or more intensive protocols may be required to achieve target values. Mean insulin levels reduced by 18% in test subjects as against 12% in controls in the Type 2 diabetes cohort, supporting the role of oral tolerance in insulin resistance. Test subjects in both cohorts reported fewer hypoglycemic episodes and reduced insulin requirement when compared with controls and with their own prestudy status. Control subjects' improvement may be due to closer monitoring and the effects of support group meetings.

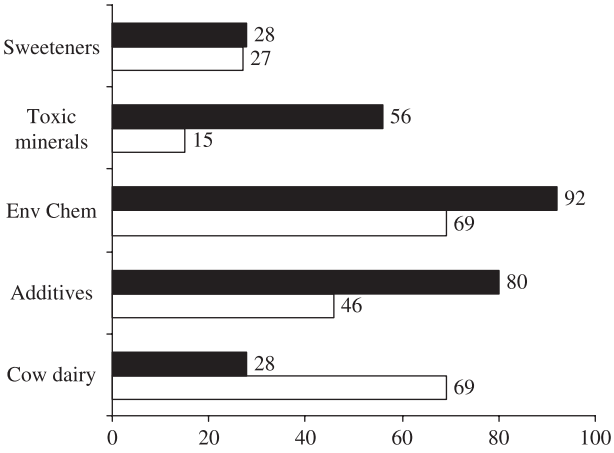

Figure 1 Comparison of immunoreactants in Type 1 (■) and Type 2 diabetics (□).

The role of oral tolerance and the immunotoxic effects of xenobiotics and anthropogenics in potentiating and maintaining the diabetic state has only recently been recognized [5]. Our findings suggest that immune reactivities to foreign antigens in diabetics are clinically important and patient specific. Immunoreactant loads may potentiate poor glycemic control and sustain diabetics in a distressed state [6]. The greater association of milk drinkers with insulin resistance in Dr Lawlor's study is consistent with our finding of much higher immunoreactivity for cow dairy among Type 2 diabetics. Immune dysregulation through a loss of oral tolerance may be an important mechanism in this regard. Reducing immunological load for the individual while providing nutrient sufficiency and neurohormonal distress reduction improved glycemic control in this study. Improved glycemic control is the goal of modern diabetes management and translates to better long-term outcomes. While larger studies are needed, we submit that comprehensive care using patient-specific functional technologies in integrated treatment plans is more cost and outcome effective than current conventional diabetes care alone.

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The functional variant –169C/T in the *FCRL3* gene does not increase susceptibility to Type 1 diabetes

Recently, Kochi *et al*. [1] reported a significant association between the *FCRL3* gene (Fc receptor-like 3) and several autoimmune diseases in Japanese subjects. The FCRL3 is a member of the Fc receptor-like family. FCRLs show high structural homology with the classical Fcγ receptors that are involved in phagocytosis, release of inflammation mediators, blood clotting, cellular cytotoxicity, immediate hypersensitivity, regulation of immunoglobulin production and immunoglobulin transcytosis. The function and the ligands of the FCRLs are not yet known, but the homology suggests that their function is similar to that of the classical receptors. Kochi *et al*. identified a single nucleotide polymorphism (SNP) in the promoter region of the *FCRL3* gene that was associated with susceptibility to rheumatoid arthritis and replicated the finding in another Japanese case–control set. The allele associated with rheumatoid arthritis also increased the risk of autoimmune thyroid disease as well as systemic lupus erythematosus. Further studies showed that this –169C/T variant in the promoter region of the *FCRL3* gene alters the expression of *FCRL3* through NF-κB binding. The higher expression was observed in individuals carrying the susceptibility allele C.

Table 1 Association analysis of the *FCRL3* –169C/T variant in patients with Type 1 diabetes

	Type 1 diabetes			
Allele/genotype	Controls	Cases	χ^2	*P*-value
T	880	868	0.033	0.856
C	582	582		
TT	262	255	0.080	0.961
TC	356	358		
CC	113	112		

Because autoimmune thyroid disease, systemic lupus erythematosus, rheumatoid arthritis and other autoimmune diseases such as Type 1 diabetes commonly cluster in the same families, it is likely that they share some genetic background, as demonstrated with the two genes *CTLA4* and *PTPN22* [2,3]. Therefore, we investigated the role of the –169C/T variant (rs7528684) in a Finnish Type 1 diabetes case–control sample from the FinnDiane study [4]. A total of 735 cases and 735 non-diabetic healthy controls were genotyped using fluorogenic 5′ nuclease allelic discrimination chemistry (TaqMan®) with an ABI Prism® 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The assay mix containing primers and probes was designed by Applied Biosystems. The overall genotyping success rate was 99%. All duplicated samples were coherent and the marker was in Hardy–Weinberg equilibrium. The diagnosis of Type 1 diabetes required age at onset of diabetes below 35 years, permanent insulin treatment initiated within 1 year of diagnosis and a fasting C-peptide level < 0.3 nmol/l. The study protocol followed the principles expressed in the Declaration of Helsinki and was approved by the local ethics committees.

We found no differences in allele frequencies between cases and controls (Table 1). The frequency of allele C was 0.398 in controls and 0.401 in cases ($\chi^2 = 0.033$; $P = 0.856$). There were no differences in genotype frequency. The SNP frequency in the Finnish control group was similar to that in the Japanese control subjects, 0.40 and 0.40, respectively. No significant differences in allele frequencies were seen with any of the measured discrete clinical parameters (sex, laser-treated retinopathy and overt diabetic nephropathy). A minor trend of the allelic association was observed with nephropathy. There were, however, no differences in genotype distribution (Table 2). Other continuous variables (age of onset of diabetes, body mass index, waist–hip ratio, HbA_{1c}) were analysed with ANOVA and no significant differences between genotypes were detected.

In conclusion, our results do not support a role for the *FCRL3* –169C/T polymorphism in the pathogenesis of Type 1 diabetes in Finnish patients.

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