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Short Communication

The prevalence of insulin autoantibodies at the onset of Type 1 diabetes is higher in males than females during adolescence

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Abstract

Aims/hypothesis. The incidence of Type 1 diabetes shows little sex bias up to age 15 years, but more males are diagnosed in early adult life. Humoral responses to the beta cell antigen insulin could help to reveal the mechanism underlying this difference. We therefore determined the influence of sex on the prevalence of insulin autoantibodies (IAA) at diagnosis.
Methods. IAA were measured by radiobinding assay in 598 patients with newly diagnosed Type 1 diabetes (aged 10.5, range 0.8–20.7 years, 333 male), and analysed according to age, sex and HLA class II genotype.
Results. Overall, 74% of males and 65% of females had IAA above the 97.5th centile of 2860 schoolchildren (p=0.028). IAA prevalence was similar in males and females under the age of 15 (0–4 yr, 95% vs 88%; 5–9 yr, 76% vs 73%; 10–14 yr, 67% vs 58%), but male excess was seen between 15 and 21 years (66% vs. 32%, p_{corr}=0.016). HLA class II genotype was available for 426 patients. IAA prevalence in DR4 homozygous patients was 87%, in DR4 heterozygous patients 72% and in DR4 negative patients 55% (p<0.001). Multivariate analysis showed independent association of IAA with age (p<0.001), number of DR4 alleles (p<0.001) and male sex (p=0.002).
Conclusions/interpretation. The prevalence of IAA in patients with newly diagnosed Type 1 diabetes is higher in males than females between 15 and 21 years of age. The lower prevalence of IAA in adolescent females implies sex-specific modulation of the autoimmune process during puberty. [Diabetologia (2003) 46:1354–1356]

Keywords Type 1 diabetes, insulin autoantibodies, sex, age, HLA.

The pattern of onset of Type 1 diabetes differs between the sexes, with earlier peak incidence in girls than boys, and a higher incidence of disease in males than females after 15 years of age [1]. The reason for this is unknown, but differences in the prevalence of the various islet autoantibodies before and after diagnosis could provide indirect clues as to the mechanisms underlying this sex difference.

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Abbreviations: IAA, Insulin autoantibodies; IA-2, protein tyrosine phosphatase IA-2; ICA, islet cell antibodies.

In a recent multinational screening study of first-degree relatives, we found a higher prevalence of islet autoimmunity in males than females during adolescence [2]. Strikingly, the prevalence of IAA did not vary up to 20 years of age in islet cell antibody (ICA) positive male relatives, whereas in female relatives the prevalence fell sharply between the ages of 10 and 20 years.

We wanted to know whether the sex difference in the prevalence of IAA observed in relatives is also seen in patients at diagnosis of Type 1 diabetes, and therefore examined the influence of sex on the prevalence and levels of IAA according to age at onset. Since there are associations between IAA and HLA class II haplotypes [3, 4], we also considered the impact of genetic factors on the prevalence of IAA in this cohort.

Subjects and methods

Subjects. Serum was obtained from 598 children and adolescents with newly diagnosed Type 1 diabetes, recruited to the Bart's-Oxford study of childhood diabetes between 1985 and 2002 [5]. Inclusion criteria were: age less than 21 years at diagnosis, insulin requirement from diagnosis, and residence in the Oxford region, United Kingdom. The median age at diagnosis of Type 1 diabetes was 10.5 years (range 0.8–20.7), and 333 were male. Samples were collected no later than 14 days after starting insulin (median 0 days, range –71 to +14 days). Blood or mouth swab samples for genetic analysis were available from 426 individuals (10.4 yr, range 0.7–20.7 yr, 242 male). The Bart's-Oxford study has been approved by the Local Research Ethics Committees.

IAA assay. Samples were assayed for IAA as previously described [5]. Results were expressed in arbitrary units derived from a standard curve. The inter-assay CV of the assay was 21% at both 0.7 units and 1.7 units. The assay achieved a laboratory-defined sensitivity of 36% with 100% specificity in the First Diabetes Antibody Standardization Program [6]. Samples were considered positive if they had levels above the 97.5th centile of 2860 schoolchildren (0.2 units).

HLA (IDDM1). HLA class II genotyping was carried out on DNA from blood or mouth swab samples. Details of DNA extraction methods and HLA class II DRB1, DQA1 and DQB1 analysis by polymerase chain reaction using sequence specific primers have been published previously [7].

Statistical analysis. Proportions were compared using Chi-square testing. When Bonferroni's correction has been applied a corrected p value is given (p_{corr}). Kendall's rank correlation was used to test for trends across groups. Multiple logistic regression was used to determine the influence of age, sex and number of HLA-DR4 haplotypes in 426 patients on the prevalence of IAA. For all analyses, a two-tailed p value of 0·05 was considered statistically significant. Analyses were carried out using the Statistics Package for Social Sciences (SPSS, Chicago, Ill., USA).

Results

The overall prevalence of IAA was 70% and did not vary with time from diagnosis; of 373 samples (218 male) taken before or on the day of diagnosis, 263 (71%) had IAA as compared to 155 (69%) of 225 samples taken in the 2 weeks following diagnosis.

IAA with age and sex. The prevalence of IAA according to age and gender is shown in Fig. 1. The influence of age was confirmed; 81% of patients under age 10 as compared to 61% of patients aged 10 to 20 years had IAA (p<0.001). Further, the prevalence of IAA fell sequentially across all four age bands. Overall, the prevalence of IAA was higher in males than females (74% vs 65%, p=0.028). The prevalence of IAA was similar in males aged 10 to 14 (67%) and 15 to 20 years (66%) (p=0.88), but fell from 58% in females aged 10 to 14 years to 32% in females aged 15 to 20 years (p=0.013).

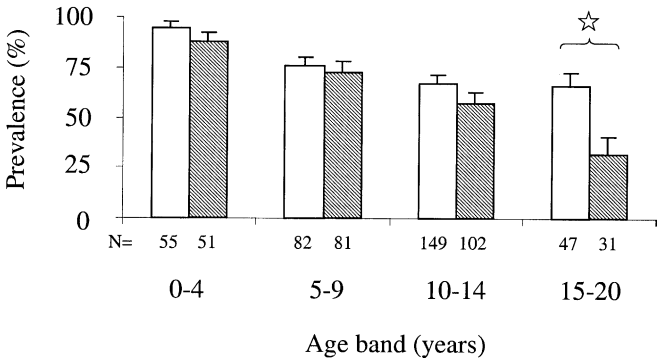

Fig. 1. Prevalence of IAA above the 97.5th centile of 2860 schoolchildren, according to age and gender, in 598 patients with newly diagnosed Type 1 diabetes. More males (*open columns*) than females (*filled columns*) aged 15–20 years had IAA (p_{corr}=0.016, *star*). The number (n) in each group is indicated below the columns. Error bars represent SE

IAA with HLA class II. As expected, IAA were associated with DR4; prevalence was highest in DR4 homozygous individuals (33 of 38, 87%), lower in DR4 heterozygous individuals (200 of 276, 72%) and lowest (62 of 112, 55%) in those negative for the DR4 haplotype (p<0.001). The prevalence of IAA in DR4 homozygous patients did not vary with age; 14 of 16 (88%) individuals under 10 years had IAA compared to 19 of 22 (86%) individuals over the age of 10 (p=0.92) and was similar in males and females (19 of 21, 90% vs 14 of 17, 82%, p=0.46). In DR4 heterozygous patients IAA were, however, more prevalent in males than females aged more than 10 years of age (61 of 82, 74% vs 24 of 51, 47% p_{corr}=0.012), despite a similar prevalence in younger patients of each sex (58 of 71, 82% vs 57 of 72, 79%, p=0.704), due to a sharp fall in older females (79% vs 47%, p_{corr}<0.006). The prevalence of IAA in DR4 heterozygous patients was not affected by DR4 subtype; of 185 carrying DRB1*0401, 130 (70%) had IAA compared to 70 of 91 (77%) carrying other DRB1*04 subtypes (p=0.24).

Multivariate analysis. The influence of age, sex and number of HLA-DR4 haplotypes on the prevalence of IAA was determined in 426 patients by multiple logistic regression analysis and showed that age (p<0.001), number of HLA-DR4 haplotypes (p<0.001) and sex (p=0.002) were independent determinants of IAA (Table 1).

Discussion

This study confirmed that IAA are inversely correlated with age in patients with newly-diagnosed Type 1

Table 1. Relative risk (95% confidence interval) for IAA in multivariate analysis

	Relative risk (95% CI)
Sex (p=0.002)	
Male	2.0 (1.3–3.2)
Female	1
Age (p<0.001)	
<5	7.6 (3.1–19.1)
5–9	2.5 (1.2–5.1)
10–14	1.5 (0.8–3.0)
15–21	1
DR4 haplotypes (p<0.001)	
2	5.5 (2.0–15.5)
1	1.9 (1.2–3.2)
0	1

diabetes [8] and that there is a strong association between IAA and HLA-DR4 [3]. The novel observation was that the prevalence of IAA in adolescents and young adult patients is strongly influenced by sex, such that only 32% of females aged 15 to 20 years had IAA as compared with 66% of males in that age group. These differences in prevalence were also reflected in the levels of insulin binding (data not shown). Multiple regression analysis showed that the male excess of IAA was not explained by differences in the number of DR4 haplotypes. Our findings contrast with those of previous studies which reported that IAA were more common in females at diagnosis [4, 9]. The difference in prevalence we observed only became apparent in adolescence and early adulthood and was seen most clearly in those heterozygous for DR4.

The age-related decline in the levels and prevalence of IAA is unexplained. Our study has also revealed a sharp fall in prevalence in females over the age of 10 which was not seen in males. In this respect IAA differed from antibodies to IA-2 and GAD; in our cohort IA-2 antibodies showed no variation with age [10], while GAD antibodies showed a positive correlation with age, and levels were higher in females. The proportions of male and female patients who were negative for both IA-2 and GAD antibodies were also similar (data not shown). These findings suggest that in females puberty may modulate autoimmunity to insulin, but not to GAD or IA-2. In our cohort the ratio of male to female patients aged over 10 was 1.5:1, despite almost identical numbers of IAA negative male and female patients in this age group. The male excess was thus due to IAA positive patients. At diagnosis, adolescent males are therefore more likely to have an autoantibody profile in which IAA predominate, resembling that of early childhood, while adolescent females tend to have a more adult profile, with higher GAD antibody levels. A similar excess of IAA was observed in male relatives of patients with Type 1 diabetes over the age of 10 in a recent screening study [2]. These observations would be consistent with continued expansion of islet autoimmunity in adolescent males and/or down-regulation of islet autoimmunity in females with puberty. Such effects, if confirmed in prospective studies, could contribute to the observed male excess of Type 1 diabetes in early adulthood.

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