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SHORT COMMUNICATION

Autoantibodies to islet antigen-2 are associated with *HLA-DRB1*07* and *DRB1*09* haplotypes as well as *DRB1*04* at onset of type 1 diabetes: the possible role of *HLA-DQA* in autoimmunity to IA-2

A. J. K. Williams · R. J. Aitken · M. A.-M. Chandler · K. M. Gillespie · V. Lampasona · P. J. Bingley

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Abstract
Aims/hypothesis To further our understanding of antigen presentation by HLA class II molecules, we have examined the influence of HLA class II genotype on expression of autoantibodies to islet antigen-2 (IA-2A).
Methods HLA class II genotype and IA-2A were determined within 3 months of diagnosis in 618 patients with type 1 diabetes (median age 11 years [range 0.7–20.9]). Antibodies to the juxtamembrane region of IA-2 were measured by a radiobinding assay in 481 of 484 IA-2A-positive patients.
Results IA-2A prevalence was highest in patients carrying at least one *HLA-DRB1*04-DQA1*0301* (385 of 450; 86%), *DRB1*07-DQA1*(0201* or *0301)* (58 of 64; 91%) or *DRB1*09-DQA1*0301* haplotype (18 of 19; 95%). Multiple regression showed that IA-2A were strongly associated with the number of these haplotypes carried; only 69 of 132 (52%) patients carrying none of these haplotypes had IA-2A, compared with 322 of 391 (82%) patients with one and 93 of 95 (98%) with two of these haplotypes ($p<0.001$). IA-2 juxtamembrane antibodies were less frequent in IA-2A-positive patients with one (35%) or two (36%) *DRB1*03-DQB1*02* or *DRB1*07-DQB1*02* haplotypes than in those negative for these haplotypes (52%) ($p=0.002$), but showed an independent positive association with IA-2A level ($p<0.001$).
Conclusions/interpretation HLA class II alleles strongly influence the prevalence of IA-2A. The high IA-2A prevalence in patients carrying *DRB1*04*, *DRB1*07* and *DRB1*09* alleles in linkage disequilibrium with *DQA1*0301* or the closely related *DQA1*0201* suggests the humoral response to IA-2 may be driven by *HLA-DQA1* genes.

Keywords HLA class II · IA-2 autoantibodies · Juxtamembrane antibodies · Type 1 diabetes

Abbreviations
IA-2A antibodies to islet antigen-2
JMA antibodies to the juxtamembrane region of IA-2

Introduction

The prevalence of antibodies to the protein tyrosine phosphatase islet antigen-2 (IA-2A) at presentation of type 1 diabetes is influenced by *HLA-DRB1*04* [1], but other genes may be important. We previously reported that IA-2A at disease onset in children and adolescents did not vary with sex or age [2], but have now determined IA-2A and *HLA-DRB1*, *-DQA1* and *-DQB1* genotype in a larger dataset of patients, allowing us to investigate the influence of these alleles on IA-2A prevalence. We have also investigated the effect of these factors on the prevalence of antibodies to the juxtamembrane region of IA-2 (JMA), which have been associated with faster progression to

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A. J. K. Williams · R. J. Aitken · M. A.-M. Chandler · K. M. Gillespie · P. J. Bingley (✉)
Diabetes and Metabolism, Clinical Science at North Bristol, University of Bristol Medical School Unit, Southmead Hospital, Bristol BS10 5NB, UK
e-mail: Polly.Bingley@bristol.ac.uk

V. Lampasona
Department of Clinical Chemistry, San Raffaele Scientific Institute, Milan, Italy

clinical disease in at-risk children [3]. Identifying the important determinants of humoral autoimmunity to this major autoantigen should improve our understanding of the pathways leading to disease, allow refinement of prediction, and may suggest new targets for immunointervention.

Methods

Type 1 diabetes

Serum was obtained from 618 children and adolescents with recently diagnosed type 1 diabetes, recruited to the Bart's–Oxford study of childhood diabetes between 1985 and 2002 [2]. All required insulin treatment from diagnosis, and were resident in the Oxford region of the UK. Median age at diagnosis was 11 years (range 0.8–20.9), and 352 were male. Samples were collected no later than 90 days after diagnosis (median 1 day) and within 30 days in 445. The study was approved by Local Research Ethics Committees.

Assay for IA-2A

Samples were assayed for IA-2A as previously described [2], using ^{35}S-labelled IA-2_{ic} (605–979). Results were expressed in arbitrary units derived from a standard curve. Samples with levels above the 97.5th percentile of 2,860 schoolchildren (0.9 units) were considered positive. The IA-2A assay had an inter-assay CV of 21% at both 0.7 and 1.7 units and achieved a laboratory-defined sensitivity of 58% with a specificity of 98% in the First Diabetes Antibody Standardization Program [4].

Assay for JMA

Binding to the juxtamembrane (JM) region of human IA-2 was investigated in 481 of the 484 IA-2A-positive samples. Samples were assayed as for IA-2A, but using a ^{35}S-labelled chimera protein encoding the JM region (609–631) of IA-2 [3]. Results were expressed as an index against a serum containing antibodies specific to the JM region. The JMA threshold was defined as the level three SDs above the mean of 80 schoolchildren. The inter-assay CV of the JMA assay was 18.4% at 0.68 units and 24.2% at 0.31 units.

Genetic analysis

HLA class II genotyping was carried out on blood or mouth swab DNA using published methods for DNA extraction and HLA class II *DRB1*, *DQA1* and *DQB1* analysis by PCR with sequence-specific primers [5].

Statistical analysis

Proportions were compared using χ^2 testing. Binary logistic regression was used to determine the influence of age, sex and HLA haplotypes on IA-2A and JMA prevalence. Age at testing was modelled in four age bands. Results for haplotype comparisons are expressed as ORs with 95% CIs in comparison with patients not carrying the respective haplotypes. The influence of IA-2A levels on JMA prevalence was investigated by modelling IA-2A levels as quartiles, with results expressed in comparison with the highest quartile. All determinants found significant at the 10% level in univariate analysis were tested together in the final model. Analyses were performed using the Statistics Package for Social Sciences Version 12.0.1 (SPSS, Chicago, IL, USA). Results were considered significant at the 5% level.

Results

In this dataset, 78% of patients had IA-2A. There was no significant variation in IA-2A prevalence with age or sex (Table 1).

IA-2A and HLA

The prevalence of IA-2A was strongly influenced by HLA genotype; prevalence was highest in patients carrying at least one *HLA-DRB1*04-DQA1*0301* (385 of 450; 86%), *DRB1*07-DQA1*(0201* or *0301)* (58 of 64; 91%) or *DRB1*09-DQA1*0301* haplotype (18 of 19; 95%). Only 69 of 132 (52%) patients carrying none of these haplotypes had IA-2A, compared with 322 of 391 (82%) patients with one and 93 of 95 (98%) with two of these haplotypes ($p<0.001$). IA-2A prevalence was similar in the 364 heterozygous patients in whom *DRB1*04* was in linkage with *DQB1*0302* and the 37 patients in whom it was in linkage with *DQB1*0301* (84 vs 89%, $p=0.39$). Further, IA-2A prevalence was similar in *DRB1*04* heterozygous patients carrying *DRB1*0401* and those carrying other *DRB1*04* subtypes (231 of 273 [85%] vs 109 of 131 [85%]).

Multiple logistic regression analysis showed that *HLA-DRB1*04* ($p<0.001$), *DRB1*07* ($p<0.001$) or *DRB1*09* haplotypes ($p=0.001$) were independent determinants of IA-2A prevalence (Fig. 1). The highest OR was seen in patients carrying two of these haplotypes in any combination (OR 42 [95% CI 10–180], $p<0.001$) (Fig. 1).

JMA

JMA were found in 202 of 481 individuals tested (42%). There was no significant variation in JMA prevalence with age or sex (Electronic supplementary material [ESM] Table 1). JMA

Table 1 IA-2A prevalence according to patient characteristics, HLA class II genotypes and *DQA1* haplotypes

Parameter	Total (*n*)	IA-2A positive		χ^2 *p* value
		n	%	
Age (years)				0.192
<5	90	75	83	
5–9	164	134	82	
10–14	273	209	77	
15–21	91	66	73	
Sex				0.794
Male	352	277	79	
Female	266	207	78	
HLA class II genotypes				
*DRB1*04-DQA1*0301/DRB1*04-DQA1*0301*[a]	46	45	98	
*DRB1*04-DQA1*0301/DRB1*07-DQA1*0201*	40	39	98	
*DRB1*04-DQA1*0301/DRB1*09-DQA1*0301*	6	6	100	
*DRB1*04-DQA1*0301/DRB1*01-DQA1*(0101* or *0501)*	66	52	79	
*DRB1*04-DQA1*0301/DRB1*03-DQA1*0501*	237	196	83	
*DRB1*04-DQA1*0301/DRB1*X*	55	47	86	
*DRB1*07-DQA1*0201/DRB1*07-DQA1*0201*	2	2	100	
*DRB1*07-DQA1*0201/DRB1*09-DQA1*0301*	1	1	100	
*DRB1*07-DQA1*0201/DRB1*01-DQA1*(0101* or *0501)*[b]	3	0	0	
*DRB1*07-DQA1*0201/DRB1*03-DQA1*0501*	12	11	91	
*DRB1*07-DQA1*0201/DRB1*X*[b]	6	5	83	
*DRB1*09-DQA1*0301/DRB1*03-DQA1*0501*	11	10	91	
*DRB1*09-DQA1*0301/DRB1*X*	1	1	100	
*DRB1*01-DQA1*(0101 or 0501)/DRB1*01-DQA1*0101*	7	6	86	
*DRB1*01-DQA1*(0101 or 0501)/DRB1*03-DQA1*0501*	32	18	56	
*DRB1*01-DQA1*(0101 or 0501)/DRB1*X*	5	3	60	
*DRB1*03-DQA1*0501/DRB1*03-DQA1*0501*	49	23	47	
*DRB1*03-DQA1*0501/DRB1*X*	36	17	47	
DRB1*X/DRB1*X	3	2	67	
Total	618	484	78	
HLA-DQA1 haplotypes				
*DQA1*0301*				
n=0	152	85	56	
n=1	414	348	84	
n=2	52	51	98	<0.001
*DQA1*0201*				
n=0	557	428	77	
n=1 or 2	61	56	92	0.007
*DQA1*0201* and/or **0301*				
n=0	132	69	52	
n=1	391	322	82	
n=2	95	93	98	<0.001

*DRB1*X* represents haplotypes not including *DRB1*01*, **03*, **04*, **07* or **09*

[a] Five of these patients were homozygous for *DQB1*0301* and 11 heterozygous for *DQB1*0301*

[b] One of these patients carried *DRB1*07-DQA1*0301*

were less common in patients with haplotypes including *DQB1*02* alleles. Only 12 of 38 (32%) IA-2A-positive patients carrying two *DRB1*03-DQB1*02* or *DRB1*07-DQB1*02* haplotypes in any combination were JMA positive and 100 of 277 (36%) with one of these haplotypes compared with 90 of 166 (54%) with none ($p<0.001$). JMA prevalence also increased with IA-2A quartile ($p<0.001$), from 31% in the lowest to 56% in the highest (ESM Table 1).

Multiple logistic regression confirmed that JMA in IA-2A-positive patients were negatively associated with *DRB1*03-DQB1*02* or *DRB1*07-DQB1*02* haplotypes (OR, two haplotypes, 0.43, 95% CI 0.2–0.92; OR, one haplotype, 0.48, 95% CI 0.32–0.71, $p=0.001$) and positively associated with IA-2A quartile (OR, 1st, 0.38, 95% CI 0.22–0.65; OR, 2nd, 0.39, 95% CI 0.23–0.66; OR, 3rd, 0.71, 95% CI 0.42–1.19, $p<0.001$).

Discussion

Our major finding was that the prevalence of IA-2A at disease onset in patients under 21 years is strongly associated

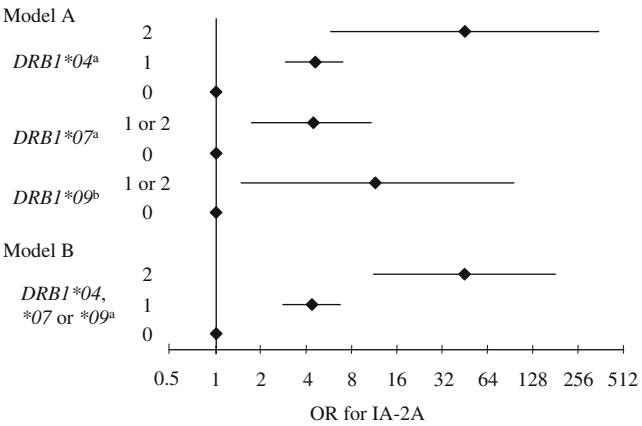

Fig. 1 ORs (diamonds) and 95% CIs (horizontal lines) for independent determinants of IA-2 antibody positivity derived from binary logistic regression analysis for 618 patients. Dependence of IA-2A prevalence on *HLA-DRB1* haplotypes as covariates was tested in Model A for patients carrying one or two *DRB1*04* haplotypes, at least one *DRB1*07* haplotype or at least one *DRB1*09* haplotype when tested together, and in model B for patients carrying one or two *DRB1*04*, *DRB1*07* or *DRB1*09* haplotypes in any combination compared with patients not carrying the respective haplotypes [a]$p<0.001$, [b]$p=0.001$

with *HLA-DRB1*07* and *HLA-DRB1*09* as well as *HLA-DRB1*04*. IA-2A prevalence was highest in patients carrying at least one *HLA-DRB1*04-DQA1*0301*, *DRB1*07-DQA1*(0201 or 0301)* or *DRB1*09-DQA1*0301* haplotype. The effect increased with the number of these haplotypes, and only two of 95 patients carrying any two of these haplotypes did not have IA-2A. Antibodies to the JM epitope of IA-2 were more common in IA-2A-positive patients with higher levels of IA-2A. JMA prevalence was also influenced by HLA class II haplotypes, being lower in those patients carrying *DRB1*03-DQB1*02* or *DRB1*07-DQB1*02*.

The *DRB1*04*, *DRB1*07* or *DRB1*09* alleles belong to the *DR53* extended haplotype [6] and share several unique characteristics, including a second expressed *DRB* locus, *DRB4*, which can play a role in presentation of autoantigens to T cells [7]. These *DRB1* haplotypes also show similarities at *HLA-DQA1*. In our patients, both *DRB1*04* and *DRB1*09* were in strong linkage disequilibrium with *DQA1*0301*, while *DRB1*07* was in strong linkage disequilibrium with *DQA1*0201*, which has close homology with *DQA1*0301*, particularly in exons 2 and 3. These exons encode the extracellular portion of the *HLA-DQA1* molecule which, in combination with *DQB1*, forms the antigen recognition site. Further, the interaction between the *HLA-DQ8* haplotype and an immunodominant insulin peptide, suggests that residues located in the P1 and P9 pockets ($Glu^{31\alpha}$ and $Ile^{72\alpha}$, respectively) shared between *DQA1*0301* and *DQA1*0201* may be important for antigen presentation [8]. Strong linkage means we cannot discriminate effects at *HLA-DRB1* and *HLA-DQA1*, but our findings are consistent with a major genetic determinant of humoral autoimmunity to IA-2 being carried by *HLA-DQA1*. Modulation of the immune response to IA-2 may therefore be an effective approach to diabetes prevention in children carrying *DQA1*0301* or *DQA1*0201* in the context of not only *DRB1*04*, but also *DRB1*07* or *DRB1*09* haplotypes. By including some genotypes currently categorised as low-risk, this strategy would be applicable to more children than one based on *DRB1*04* haplotypes alone.

IA-2 antibodies have been associated with *DRB1*09* in the Japanese [9], but this allele is uncommon in Europeans. The strong association of IA-2A with *DRB1*07* haplotypes in patients with type 1 diabetes has not been recognised previously, possibly because of their lower frequency in comparison with *DRB1*04* haplotypes. Indeed, *DRB1*07* haplotypes are considered neutral or protective in the UK population [5]. Of our patients, only 12 had the genotype *DQA1*0201-DQB1*0201 (DRB1*07)* combined with *DQA1*0501-DQB1*0201 (DRB1*03)*, but 11 (92%) of these had IA-2A despite being associated with a lower prevalence of multiple antibodies and diabetes risk in relatives [10]. In contrast, only 23 of 49 (47%) patients homozygous for *DRB1*03* haplotypes had IA-2A ($p=0.008$) despite the much higher diabetes risk associated with this genotype [5].

The lower prevalence of antibodies directed to the JM epitope in IA-2A-positive patients carrying *DQB1*02* is unreported. *DQB1*02* is carried on *DRB1*03* and **07* haplotypes. Presumably, increased prevalence of IA-2A in patients with *DRB1*07* haplotypes is because of antibodies directed mainly to the other major epitopes of IA-2 found in the protein tyrosine phosphatase domain [3]. The increased JMA prevalence at higher IA-2A levels suggests increased epitope spreading in those with a more vigorous humoral response.

In conclusion, HLA class II alleles strongly influence humoral autoimmunity to IA-2 in patients at disease onset. IA-2A prevalence was highest in patients carrying *DQA1*0301* or the closely related *DQA1*0201*, suggesting that the antibody response to IA-2 may be determined by *DQA1* alleles.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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