*For debate***A model for the involvement of MHC class II proteins in the development of Type 1 (insulin-dependent) diabetes mellitus in response to bovine serum albumin peptides****B. H. Robinson¹, H.-M. Dosch¹, J. M. Martin¹, H. K. Åkerblom³, E. Savilahti², M. Knip⁴ and J. Ilonen⁵**¹ Departments of Biochemistry, Immunology and Paediatrics, The Research Institute, Hospital for Sick Children and The University of Toronto, Toronto, Ontario, Canada; ² Childrens Hospital, I Department of Paediatrics, University of Helsinki, Finland;³ II Department of Paediatrics, University of Helsinki, Finland; ⁴ Department of Paediatrics, University of Oulu, Finland;⁵ Department of Virology, University of Turku, Finland

The predisposition of an individual to develop Type 1 (insulin-dependent) diabetes mellitus is strongly influenced by the MHC class II proteins expressed by that individual particularly those of DR and DQ. Genetic susceptibility to Type 1 diabetes is as a result determined by the structure of the α - and β -chains of DQ and the β -chain of DR [1]. Since the classic demonstration by McDevitt and colleagues [2] that aspartate at position 57 of the DQ β -chain was protective against the development of Type 1 diabetes, many populations have been surveyed to see how the incidence of Type 1 diabetes varies with DR and DQ haplotypes. While the basic tenet of the hypothesis that having non-⁵⁷Asp/non-⁵⁷Asp alleles at DQ β predisposes to Type 1 diabetes has proved to be true for Caucasian populations, it has become apparent that often the accompanying DQ α or DR β chains may have a modifying effect [3]. The mechanism for the genetic susceptibility to Type 1 diabetes has been the subject of much debate with two somewhat different models emerging.

In the model first proposed by Nepom [4], the array of MHC class II dimers encoded in any one individual vary in their affinity for the antigenic peptide that can induce autoimmunity to a protein present in the beta cells of the islets of Langerhans. Class II dimers which confer susceptibility promote this autoimmune reaction by strongly binding the antigenic peptide, much more strongly than class II dimers which may confer non-susceptibility. Conversely, Sheehy [5] proposed a model in which the non-susceptibility class II alleles promote tolerance to the self-protein or peptide of the beta cells by binding that peptide. Susceptibility to autoimmune disease results because a weak or non-existent binding of the self-peptide by MHC class II proteins bestows a lack of tolerance to the self-protein.

Following evidence that in Type 1 diabetes there is a strong immune reaction to cow's milk proteins [6] we proposed that a peptide ADEKKFWGKYLEIARRH (ABBOS peptide) present in bovine serum albumin (BSA) was responsible for the autoimmune response directed against islet cells [7]. Recently we obtained convincing evidence that a strong immune reaction was directed against this peptide in diabetic patients at the time

of diagnosis [8, 9]. Here we use the structure of this candidate peptide antigen to explore possible interactions with MHC class II proteins. This exercise gives some simple explanations of allele susceptibility and suggests a new model for the development of Type 1 diabetes in terms of MHC class II involvement.

Involvement of ABBOS-peptide antigen in the autoimmune process

A diet free from cow's milk early in life prevents diabetes in BB rats and early BSA immunization accelerates while tolerisation with BSA prevents the disease [6, 9–11]. Breast feeding and delayed exposure to cow's milk based formulas has been correlated with reduced incidence of Type 1 diabetes [12–14], and countries with a high per capita cow's milk consumption show a trend towards a high incidence of Type 1 diabetes [13, 15]. The antigenicity shown in newly-diabetic rats, mice and children toward cow's milk proteins [8, 9, 16–18] was shown to be most selective toward the BSA present in milk [8, 9]. In 142 newly-diagnosed Type 1 diabetes patients from Finland all had elevated levels of rapidly binding BSA antibodies with the bulk of this response mapped to the ABBOS peptide [9]. Newly-diagnosed subjects with Type 1 diabetes showed strong T-cell proliferative responses to BSA, with epitope specificity mapping to ABBOS (Dosch et al., unpublished observations). Demonstration of a γ -interferon inducible islet beta cell protein of M_r 69 kDa (p69) cross-reactive with antibodies to BSA and ABBOS [7–9, 19] have led to a model in which early exposure to cow's milk via immature gut permeability triggers an immune response to BSA that slowly over a period of years results in islet cell destruction, a process spurred on by viral infection.

Correlation of MHC class II predisposition specificity for Type 1 diabetes development and the predicted binding characteristics of the ABBOS peptide

The third hypervariable region of HLA class II β subunits DR and DQ have a charge structure (⁵⁷ $\underline{n}X - X^5 + -X^5 + X \pm$) (where $\underline{n}$ is either neutral or negative) which

Table 1. Alignment of Type 1 diabetes susceptible and non-susceptible DQ allotypes with the reverse ABBOS sequence. The amino acids for DQ β sequence are listed for positions 50–79. Possible charge attraction, repulsion or misalignment is considered for posi-

tions 57, 59, 66, 70, 71, 72, 74 and 77 indicated with * below in relation to the anteverted ABBOS peptide. An overall score is calculated from the resultant of the potential for alignment by charge attraction

	55	60	65	70	75	Repel or Misalign	Attract	Overall Score	Diabetes Association
Charge	n	-	+ -	- ± ± +	- - +				
DQB*0302	: P P A A E Y W N S Q K E V L E <u>R</u> T R A <u>E</u> L D T V C					-2	+3	+1	Positive ++
DQB*0201	: L P A A E Y W N S Q K D I L E <u>R</u> <u>K</u> R A A V D <u>R</u> V C					-2	+4	+2	Positive ++
DQB*0501	: R P V A E Y W N S Q K E V L E G A R A S V D <u>R</u> V C					0	+4	+4	Positive +
DQB*0604	: R P V A E Y W N S Q K E V L E <u>R</u> T R A <u>E</u> L D T V C					-2	+3	+1	Positive +
DQB*0502	: R P S A E Y W N S Q K E V L E G A R A S V D <u>R</u> V C					0	+4	+4	Positive +
DQB*0301	: P P D A E Y W N S Q K E V L E <u>R</u> T R A <u>E</u> L D T V C					-2	+4	+2	Neutral/Negative
DQB*0601	: R P D A E Y W N S Q K D I L E <u>R</u> T R A <u>E</u> L D T V C					-2	+4	+2	Neutral/Negative
DQB*0503	: R P D A E Y W N S Q K E V L E G A R A S V D <u>R</u> V C					0	+5	+5	Neutral
DQB*0602	: R P D A E Y W N S Q K E V L E G T R A <u>E</u> L D T V C					-1	+4	+3	Negative
DQB*0603	: R P D A E Y W N S Q K E V L E G T R A <u>E</u> L D T V C					-1	+4	+3	Neutral/Negative
DQB*0401/402	: R L D A E Y W N S Q K D I L E <u>E</u> <u>D</u> R A S V D T V C					0	+6	+6	Neutral/Positive
Rev-ABBOS	: Y P H R R A I E Y L Y K G W F <u>K</u> <u>K</u> E D A K F <u>E</u> D C								
	*	*	*	*	*				

Table 2. Predisposition to Type 1 diabetes in DR haplotypes in relation to charge affinity for the reverse ABBOS sequence. The amino acids of the third hypervariable region of the β -chain encoded by different DR4 and DR7 allotypes are illustrated as for the previous figure, highlighting residues that are likely to cause attraction, repulsion or misalignment with the anteverted ABBOS sequence (RAB-BOS)

	57	65	70	71	Type 1 Diabetes Association
DR7:	V A <u>E</u> S W N S Q K <u>D</u> I L E <u>R</u> <u>R</u> G Q V D				Negative
DRB1*0401:	<u>D</u> A E Y W N S Q K <u>D</u> L L E Q <u>K</u> R A A V D				Positive
DRB1*0402:	D A E Y W N S Q K <u>D</u> I L E <u>R</u> <u>R</u> A A V D				Positive
DRB1*0403:	D A E Y W N S Q K <u>D</u> L L E Q <u>R</u> R A <u>E</u> V D				Negative
DRB1*0404:	D A E Y W N S Q K <u>D</u> L L E Q <u>R</u> R A A V D				Neutral
DRB1*0406:	D A E Y W N S Q K <u>D</u> L L E Q <u>R</u> R A <u>E</u> V D				Negative
DRB1*0407:	D A E Y W N S Q K <u>D</u> I L E <u>R</u> <u>R</u> A A V D				Positive
Rev-ABBOS:	<u>H</u> R <u>R</u> A I E Y L Y <u>K</u> G W F <u>K</u> <u>K</u> E D A K F				
0402 > 0401 > 0404 > 0403 = 0406					
Strong.....Weak					
Association with Type 1 Diabetes					

has a symmetry of charge distribution that would theoretically allow an anteverted peptide to bind to the parent molecule efficiently (Table 1). Information from both X-ray crystallography [20] and from theoretical computer modelling of peptide fitting [21] strongly suggests that MHC proteins that present peptides on the cell surface do so in an orientation that has the N-terminus of the peptide aligned with the C-terminal end of the third hypervariable region of MHC class II protein β -subunits.

Table 1 illustrates such an anteverted alignment of residues for the ABBOS peptide and the DQ allotypes predisposing for and against Type 1 diabetes. Consideration of the possibilities for binding through oppositely charged residues suggests that β -chains encoded by DQB*0401 and DQB*0602 would bind better than DQB*0201 and DQB*0302, the latter actually showing charge repulsion by ^{70}Arg for DQB*0302 and by ^{70}Arg ^{71}Lys for DQB*0201. The presence of ^{74}Glu would also

weaken binding by promoting misalignment. The C-terminus of the ABBOS peptide would be anchored by attraction of a histidine residue for ^{57}Asp . This peptide specificity is not very suggestive of an involvement of DQ ^{57}Ala as the binder of the ABBOS peptide and therefore makes it unlikely that the DQ $^{57}\text{Ala}/^{57}\text{Asp}$ discrimination for Type 1 diabetes is simply at the level of presentation of the BSA peptide by DQ.

On the other hand, an anteverted alignment of the BSA peptide with DR β subunit third hypervariable regions, by similar reasoning suggests that the most efficient binding would be by the ^{57}Asp alleles (Table 2). On the contrary, binding and presentation of ABBOS would be strengthened by allotypes possessing neutral or negatively charged residues at positions 70 or 71. Sheehy [5] points out that in those haplotypes bearing DR4 and DQw8 there is considerable variability in susceptibility to diabetes. Thus β -chains encoded by DRB*0402 and

Fig.1. Scheme to show the course of development of Type 1 diabetes in a susceptible and non-susceptible host. In this model the antigen present on the surface of the islet beta cells is presented by DQ. Poor presentation is evident when the subject has ⁵⁷Asp negative alleles for DQβ and good presentation results when alleles for DQβ are ⁵⁷Asp positive. The foreign antigen ABBOS is presented by

DR, well by ⁵⁷Asp positive DRβ but poorly by ⁵⁷Asp negative DRβ. The overall predisposition to diabetes is determined by the ABBOS and islet antigen binding affinities of DR and DQ and the interplay between the strength of self recognition and the immune response to the foreign protein epitope

DRB*0407 which have the strongest charge affinity for ABBOS also have strong associations with Type 1 diabetes. This is because positions 70 and 71 are occupied by aspartate and glutamate respectively and provide a perfect attractive alignment for the double lysine of ABBOS. At the other end of the scale, having glutamine and arginine at positions 70 and 71 appears to be protective against diabetes in 0406 and even more so in 0403 when a glutamate is in position 74 instead of the usual alanine [3, 22]. In fact the ⁷⁴Glu would tend to favour the alignment of the residues ⁷¹Arg ⁷²Arg with the Asp Glu of the ABBOS sequence thus causing poor alignment of the C-terminal end of the peptide. Lysine at position 71 as in DRB*0401 does not seem to endow as much protection against diabetes as arginine at position 71 perhaps because of the slight difference in charge or more likely because of the difference in bulk of the side chain. The β-chains of DR7, DR8 and DR9 all have this ⁷¹Arg and a decreased Type 1 diabetes association present with a non-⁵⁷Asp DQ allotype. Thus they also would have decreased ability to bind ABBOS because of charge repulsion at ⁷¹Arg and lack of charge attraction at ⁵⁷Val or ⁵⁷Ser.

A model for Type 1 diabetes that incorporates both DR and DQ specificities and antigenicity of the ABBOS peptide

Figure 1 shows a sequence of events which could lead to development of Type 1 diabetes. Initially the process of immune recognition of the hypothetical p69 self-membrane antigen is considered. For the purposes of this discussion we assume that p69 carries a homologous peptide to ABBOS for which there is compelling evidence [7–10]. If we consider the binding of the p69/ABBOS-like peptide by DQ we would predict that it would be presented poorly by individuals with non-⁵⁷Asp/non-⁵⁷Asp allotypes but it would be well presented by ⁵⁷Asp DQ endowed individuals. Furthermore, we would predict that DQ haplotypes with ⁷⁰Arg or ⁷¹Lys would be the weakest presenters of the hypothetical membrane peptide. This would make the β-chain encoded by DQB*0201 the poorest presenter of the membrane antigen followed by DQB*0302 and DQB*0604. Chains encoded by DQB*0501 and DQB*0502 would have somewhat stronger binding and might be relatively spared in susceptibility to Type 1 diabetes. This process of membrane antigen presentation

would therefore give poor tolerance to non-⁵⁷Asp homozygous individuals and strong tolerance to ⁵⁷Asp positive individuals. The strong protective effect of the β -chain of DQB*0602 would be predicted from the absence of charged residues in the 70 and 71 positions, while the poor binding characteristics of DQB*0301 and DQB*0601 suggest an explanation for their increase in ⁵⁷Asp positive subjects with Type 1 diabetes [22].

Binding of the foreign peptide ABBOS derived from BSA in cow's milk in this model is by DR in most individuals preferentially by ⁵⁷Asp containing DR allotypes (see previous section). The predisposition to develop Type 1 diabetes based on the identity of MHC class II proteins is a dynamic between self-recognition of the islet beta cell membrane antigen peptide and the strength of the immune response to the ABBOS peptide presented by DR. This presentation of ABBOS peptide is particularly apt to take place in the intestine where surface epithelial cells express DR antigens, while the DQ expression is either absent or weak [23].

This model explains several points which have only been covered partly in the models proposed by other investigators. Firstly it explains the protection conferred by the ⁵⁷Asp DQ haplotypes. A tolerisation model fits this piece of information much better than one in which DQ is responsible for presenting the foreign antigen. Secondly it explains why the non-⁵⁷Asp/non-⁵⁷Asp DQ individuals are the ones most susceptible to developing Type 1 diabetes. Thirdly it offers a comprehensive explanation for the strong modifying influence of DR allotype in the presence of Type 1 diabetes predisposing DQ allotypes. Fourthly it explains why DR and DQ alleles differing only at positions 57, 70, 71 and 74 of the third hypervariable regions can have such different effects in Type 1 diabetes susceptibility. Lastly and most convincingly, it provides a simple and logical explanation for the fact that individuals with mixed DR3/DR4 haplotypes are the most susceptible to Type 1 diabetes. Since DQB*0201 and DQB*0302 have the poorest theoretical binding protein for the islet membrane antigen and DRB1*0402 has the strongest theoretical binding allotype for the ABBOS peptide, it follows that the most susceptible individuals will be MHC class II heterozygotes with the mixed DRB1*0301.DQB*0201 and DRB1*0402.DQB*0302 haplotypes. However, since the DRB1*0402 allele is primarily present only in Jewish populations, in most countries other high affinity DR4 alleles such as DRB1*0401 associated with DQB*0302 predominate.

⁵⁷Asp positive DQ allotypes present in Type 1 diabetic patients

A further point that needs to be addressed in the light of this hypothesis is the anomalous behaviour of DQB*0401/402 and its strong representation in populations exhibiting Type 1 diabetes with ⁵⁷Asp/non-⁵⁷Asp DQ heterozygotes [24–26]. According to the hypothesis so far advanced DQB*0401/402 should be the best presenter of the p69/ABBOS-like peptide derived from islet membranes and thus very protective. The third hypervariable

region of these two alleles (401/402) is identical and resembles DRB1*0402 and DRB1*0407 in having aspartate at position 57 and glutamate and aspartate at positions 70 and 71, respectively. These alleles occur in cis or trans combination with DQA1*0301 on haplotypes often bearing the non-⁵⁷Asp DR alleles DRB1*0801 or DRB1*0405 [26, 27]. The non-⁵⁷Asp DQ allele is most commonly DQB*0302. We suggest that when such haplotypes are present together the roles are switched such that DQ becomes the presenter of the ABBOS peptide and DR the presenter of the membrane peptide. A double non-⁵⁷Asp DR β -chain when present ensures poor presentation of the islet membrane antigen, while the $\alpha\beta$ -chain combination encoded by DQA1*0301.DQB*0401/402 binds the ABBOS peptide efficiently and secures a strong immune response. The alternative explanation of the lack of protection offered by DQB*0401/402 lies in the presence of the ⁵²Arg containing α -chain encoded by DQA1*0301 in DQw4 haplotypes [3, 5, 22, 27]. The presence of ⁵²Arg in the α -subunit may in fact have a dramatic effect on the structure of the $\alpha\beta$ DQ dimer and its binding characteristics so that the normal high affinity for the islet membrane antigen is drastically modified. This might be a more satisfactory explanation than role switching of DQ in these ⁵⁷Asp positive individuals but both explanations are equally plausible with the evidence available at present.

Conclusions

Considerations of the structure of a candidate initiating antigen have led us to propose a new model for the susceptibility to Type 1 diabetes. It is quite likely there are other antigens of similar structure of viral or bacterial origin that will provoke a similar response directed against islet beta cells. However, we believe that this hypothesis will provide a framework for the further investigation and research into the mechanism of development of Type 1 diabetes and for the design of therapeutic manoeuvres for its prevention.

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