operatively, and, in particular, for localising recurrences or metastases of medullary thyroid carcinomas.

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Association of specific immune response to pork and beef insulin with certain HLA-DR antigens in type 1 diabetes

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Abstract

To test the association of HLA-DR antigens with high-responder and low-responder status to either beef or pork insulin, insulin antibodies in diabetic sera were separated into those with average low and those with average high affinity and their insulin-binding capacities for each insulin determined. Significantly less binding of pork insulin by the high affinity antibodies occurred in the group of patients with DR3 antigens compared with those with DR4 antigens ($p < 0.01$) and DR3/4 antigens ($p < 0.01$). The difference in the binding capacity of beef insulin by the high affinity antibodies between the groups with DR3 and DR4 antigens was less pronounced but still significant.

The high-responder status of DR3/4 antigens to pork insulin suggests that the gene or genes associated with HLA-DR4, and responsible for a high response to pork insulin, are dominant to genes associated with HLA-DR3 and a low response. If extended to human insulin and different HLA-DR and HLA-B antigen patterns, these findings should help in the therapeutic selection of the appropriate insulin and thus reduce the induction of an anti-insulin response in patients with diabetes.

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Introduction

The immune response to insulin is under the control of genes, the so-called immune response genes, which are located within the major histocompatibility complex. Immune response genes to insulin have been most convincingly demonstrated in mice and guinea pigs, whereas their investigation in humans is far less advanced. Schernthaner *et al*[1] and Bertrams *et al*[2] correlated the high responder status of antibody production to insulin with the HLA-DR4 and B15 antigens, while DR3 and B8 antigens were more closely associated with a low responder status. Their particular technical approach did not allow discrimination between a high responder and low responder status with respect to the most commonly used insulins, beef and pork.

The insulin molecule is small and carries on its surface only a limited number of antigenic determinants. Furthermore, beef insulin differs from human insulin by three amino-acid exchanges: Ala for Thr at A8 (A-chain loop region), Val → Ile at A10, and Ala → Thr at B30 (B-chain). Pork insulin differs only by one exchange: Ala → Thr at B30. To detect an antibody with binding specificity for one of these sites it is important that the antigen in the assay retains its native structure as far as possible. If the antibody-bound and free insulin is, however,

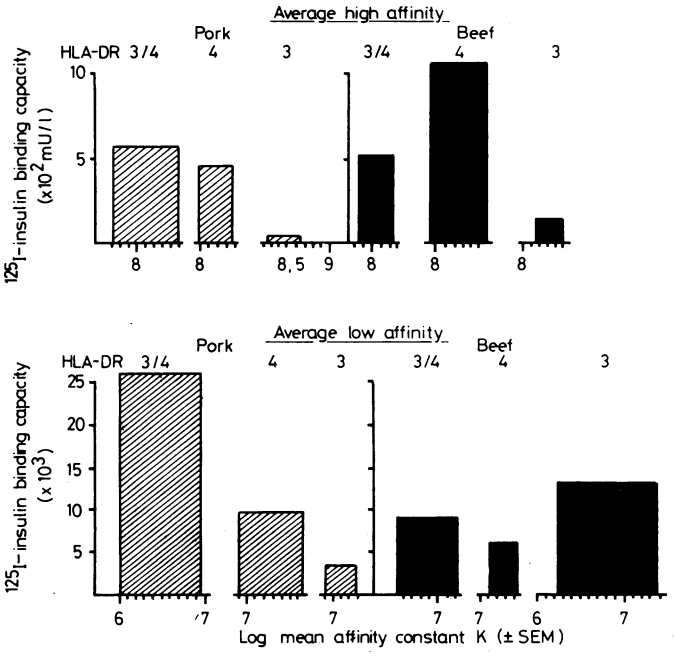
separated using a solid or semisolid phase radioimmunoassay, adsorption to the solid phase could lead to a distortion or unavailability of some antigenic structure. We used a liquid phase radioimmunoassay utilising the polyethylene glycol precipitation of the insulin-antibody complexes.

The distribution and prevalence of antibodies with different affinities to a given antigen is expected to vary between individuals.[3] This distribution can only be assessed using binding curves in contrast to single-point binding data. The binding curve is a straight line in a double reciprocal plot if a homogeneous population of antibodies is present; however, this is true only in a limited number of patients with diabetes.

In this study we tested the hypothesis that if the insulin antibodies in diabetic sera are separated into those with average low and average high affinity and their total insulin binding capacities for a given insulin determined the DR antigens can be directly correlated with high-responder or low-responder status to either beef or pork insulin.

Materials, methods, and results

The total binding capacities of eight DR3/4, eight DR4, and eight DR3 insulin-dependent (type 1) diabetics who were HLA-typed according to the standard microlymphocytotoxicity test were compared.[4] The polyethylene glycol precipitation method (PEG 6000, Merck Laboratories) was used to separate the bound and free ^{125}I-labelled pork and beef insulin, specific radioactivity 23-30 μCi/μg (Novo Research Institute).[5] With each serum two pairs of binding curves were performed, one for each of the insulin species. Each pair consisted of one binding curve at a small antigen concentration ($0{\cdot}5\text{-}17\times10^{-9}$/mol), which preferentially detected the high affinity antibodies, and one at a larger antigen concentration ($3\text{-}130\times10^{-9}$/mol) for the low affinity antibodies. The overall insulin concentration range examined was over 200-fold. The total insulin binding capacities and the equilibrium constant K were calculated after subtraction of non-specific binding using the Langmuir reciprocal plot and the logarithmic transformation of the Sips equation.[6 3] Thus, for each insulin species a total binding capacity of an average lower antibody affinity (K value range = 10^5-10^7 l/mol) using the larger antigen concentrations and of an average higher affinity (K = 10^7-10^9 l/mol) using the smaller antigen concentrations was obtained. The division into these two average affinity compartments was justified by the finding of different slopes in the Langmuir plot as well as in the Sips plot comparing the two binding curves. This is also in agreement with other workers who described bimodal and other distributions of antibodies affinities.[3]



Comparison of the total binding capacities of pork and beef insulin in HLA-DR3/4 (n=8), DR4 (n=8), and DR3 (n=8) type 1 diabetics. The bar height=arithmetical mean of ^{125}I-Insulin bound in mU/l (× factor 7·175 to obtain pmol/l). (Statistical differences: between average high affinity antibodies; (pork)—HLA-DR3/4 *vs* HLA-DR4, not significant; HLA-DR3/4 *vs* HLA-DR3, $p<0{\cdot}01$; HLA-DR4 *vs* HLA-DR3, $p<0{\cdot}01$; (beef)—HLA-DR3/4 *vs* HLA-DR4, not significant; HLA-DR3/4 *vs* HLA-DR3, not significant; HLA-DR4 *vs* HLA-DR3, $p<0{\cdot}05$; between average low affinity antibodies; (pork)—HLA-DR3/4 *vs* HLA-DR4, not significant; HLA-DR3/4 *vs* HLA-DR3, $p<0{\cdot}05$; HLA-DR4 *vs* HLA-DR3, $p<0{\cdot}05$; (beef)—HLA-DR3/4 *vs* HLA-DR4, not significant; HLA-DR3/4 *vs* HLA-DR3, not significant; HLA-DR4 *vs* HLA-DR3, not significant.)

Since the total insulin binding capacities (mU/l) were not normally distributed, the different HLA-DR groups were compared using the Wilcoxon-Mann-Whitney rank sum test. Significantly less binding of pork insulin by the high affinity antibodies occurred in the DR3 group compared with the DR4 group ($p<0{\cdot}01$) and the DR3/4 group ($p<0{\cdot}01$) (figure). In the average lower affinity compartment the difference was also found (DR3 *vs* DR4, $p<0{\cdot}05$; DR3 *vs* DR3/4, $p<0{\cdot}05$). The difference of high-affinity antibody binding of beef insulin between the DR3 and DR4 groups was less pronounced compared with the pork insulin binding; it was, however, still statistically significant ($p<0{\cdot}05$). No difference was present in the lower affinity compartment for beef insulin between the DR3 and DR4 groups. There was no significant difference in either compartment in the binding of beef insulin between the DR3 compared with the DR3/4 groups. Although a trend to increased pork insulin binding was found in the DR3/4 group compared with the DR4 group, this did not reach statistical significance with the patient sample studied (DR4, n=8; DR3/4, n=8).

All patients were treated with monocomponent insulins for at least two years. The mean values ± SD of the current insulin treatment (U/kg body weight) for the group DR3/4, DR4, and DR3 were $0{\cdot}671\pm0{\cdot}184$, $0{\cdot}517\pm0{\cdot}173$, and $0{\cdot}665\pm0{\cdot}275$ with a non-significant difference using analysis of variance. All the patients included were immunised with beef in addition to pork insulin by means of treatment in the past, as confirmed by the clinical records or the binding data or both.

The few patients who presently receive beef insulin in addition to pork insulin are in the groups DR3/4, four out of eight; DR4, four out of eight; and DR3, two out of eight.

Discussion

The present data, which were collected from a restricted number of patients with diabetes, indicated that DR3 carriers have a low-responder status to pork but not to beef insulin. DR4 and DR3/4 carriers have a high-responder status to beef as well as to pork insulin. Particularly interesting is the high-responder status of DR3/4 to pork insulin, as it suggests that the gene or genes associated with HLA-DR4 and responsible for a high response to pork insulin are dominant to genes associated with HLA-DR3 and a low response. In the animal model the insulin immune response genes are usually dominantly inherited, which might be analogous to these data.

These findings support the view that immune response genes controlling the anti-insulin response are also operational in humans. Furthermore, the association of the immune response genes and the DR alleles and the difference between DR3 and DR4 support the notion of the genetic and immunological heterogeneity of insulin-dependent (type 1) diabetes as suggested by the National Diabetes Data Group (National Institute of Health, USA).

The humoral anti-insulin response has been put forward as one cause which can aggravate development of diabetic retinopathy.[7] Some studies suggest that the presence of HLA-DR4 in insulin-dependent (type 1) diabetics is associated with a greater susceptibility to retinopathy.[8]

We suggest that if this study is extended to human insulin and different HLA-DR and HLA-B patterns it should help in selecting the appropriate insulin for treatment and thus reduce the induction of an anti-insulin response in diabetics. Moreover, study of the responder status to the three insulin species mentioned could help to subclassify further insulin-dependent (type 1) diabetes.

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Decreased plasma motilin concentrations in pregnancy

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Abstract

Plasma motilin concentrations were measured in 37 women during the second and third trimester of pregnancy and one week after delivery. The mean plasma motilin concentrations, both fasting and after a glucose load and a mixed meal, were significantly ($p<0.001$) reduced during pregnancy, returning to the normal range one week post partum. Pregnancy appears to have a profound inhibitory effect on plasma motilin, and this may in part be responsible for the gastrointestinal hypomotility associated with pregnancy.

Introduction

There has been considerable interest recently in the effect of pregnancy on gastrointestinal motility.[1-3] Clinical observations such as heartburn, constipation, and gall bladder stasis have been variably attributed to the muscle-relaxing effects of progesterone and the physical impediment of the uterus. Progesterone reduces lower oesophageal sphincter pressure and gastrointestinal motility and also delays gall bladder emptying.[4-6] It is still not certain, however, whether these effects are direct or mediated via the action of a gastrointestinal hormone.

Motilin is a hormonal peptide[7] that has gastrointestinal smooth-muscle stimulating effects. Its target organs include the lower oesophageal sphincter,[8] the stomach,[9 10] small[11 12] and large intestine,[13] and the gall bladder.[14 15] We investigated the possibility that motilin secretion is altered in pregnancy.

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Subjects and methods

Three groups of patients were recruited before 16 weeks' gestation to study the effect of maternal adiposity on glucose tolerance during pregnancy.[16] A total of 37 subjects were studied, nine whose weight was less than 80% of their ideal body weight, 10 whose weight was between 90 and 110% of their ideal body weight, and 18 with a weight greater than 120% of ideal body weight. These women were studied at 16 and 36 weeks of pregnancy and one week post partum. After an overnight fast the subjects were given a conventional three-hour 50-g oral glucose tolerance test and half-hourly blood samples were taken via an indwelling intravenous cannula. After this the subjects were given a mixed meal of about 2·09 MJ (500 calories), containing 40% fat, 40% carbohydrate, and 20% protein, and further half-hourly blood samples were taken over a period of three hours. The blood samples were centrifuged immediately and the plasma stored at −20°C.

Details of the plasma motilin radioimmunoassay used have been published.[7 9] The antibody used (M1) does not crossreact with any of the known gut hormones, and appears to be *N*-terminal specific. This assay can detect changes in plasma motilin of 3 pmol/l with 95% confidence. To avoid interassay variation, all the samples in the present study were estimated in one assay (intra-assay variation was 5%). The mean fasting motilin concentration (±SEM) observed in normal non-pregnant subjects in this laboratory is 48±6 pmol/l.[7]

The research protocol was approved by the ethics committee of St Mary's Hospital and informed consent was obtained from each subject before the study.

The mean and standard error of the mean (mean±SEM) have been used throughout. The Wilcoxon signed rank test was used to test for differences in the motilin concentrations at 16 and 36 weeks of pregnancy and post partum. The mean of the values obtained 30 minutes before and immediately before the glucose load have been taken as the mean fasting value. The mean of the values 150 and 180 minutes after the glucose load have been taken as the "basal" value before the meal.

Results

There was no difference in the plasma motilin concentrations in the groups studied and no correlation between body weight and motilin concentrations; the data from the three groups have therefore been pooled.

The figure shows the results obtained. The mean fasting plasma motilin concentration at 16 weeks of pregnancy was 23±2·8 pmol/l (median 20, range 5-91 pmol/l). This was not significantly different from the concentration at 36 weeks of pregnancy (21±2·5 pmol/l, median 17, range 4-58 pmol/l). The concentration one week after