Islet cell surface antibodies in genetically obese hyperglycaemic (ob/ob) mice

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A quantitative method for circulating islet cell surface antibodies (ICSA), based on the binding of ¹²⁵I-protein A to insulin-producing RINm5F cells, was used to evaluate ICSA in plasma of 4- to 40-week-old Aston obese hyperglycaemic (ob/ob) mice and normal control (+/+ ) mice. RINm5F cells bound 2502 ± 1196 c.p.m. ¹²⁵I-protein A per 10⁶ cells (mean ± S.D., n = 54) after incubation with +/+ plasma. ICSA positive plasma (defined as ¹²⁵I-protein A binding, mean ± 2 S.D. of +/+ plasma) was detected in 3 out of 54 +/+ mice and 3 out of 54 ob/ob mice. ICSA were not observed in ob/ob mice before the onset of diabetes (7 weeks of age), but were detected at 9, 20 and 40 weeks. At 20 weeks ¹²⁵I-protein A binding produced by ob/ob plasma was 35% greater than +/+ plasma (P < 0.05). The low occurrence of ICSA in ob/ob mice (6%) suggests that factors other than ICSA are responsible for B-cell dysfunction and eventual islet degeneration observed in Aston ob/ob mice.

Raised circulating titres of islet cell surface antibodies (ICSA) have recently been noted in several syndromes of hypoinsulinaemic diabetes. These include idiopathic type 1 diabetes in man (1,2) and spontaneous syndromes of diabetes in Bio Breeding (BB) rats (3, 4), non-obese diabetic (NOD) mice (5) and macaque monkeys (Macaca nigra) (6). Raised ICSA shortly precede or coincide with the onset of islet B-cell dysfunction and degeneration, often associated with insulitis. The ICSA do not necessarily persist after the appearance of diabetes, and may even arise only after the development of hyperglycaemia (7). In the human and animal syndromes studied, not all conditions with raised ICSA result in diabetes, and not all cases of diabetes show raised ICSA. However, available evidence suggests that ICSA may contribute to the pathogenesis of B-cell disruption in susceptible individuals (7-10).

ICSA have not been examined in obese, insulin resistant syndromes of diabetes which exhibit B-cell dysfunction with progression to islet degeneration in later life. In the present study ICSA were measured using the ¹²⁵I-
protein A radioligand assay at frequent intervals during the development of diabetes and during ageing in Aston obese hyperglycaemic ob/ob mice and normal controls.

Materials and Methods

Groups of Aston ob/ob mice and normal control (+/+) mice were reared and maintained as described previously (11, 12). Blood samples (60 µl) were taken from the tail tip of non-fasted conscious mice between 4 and 40 weeks of age as shown in the figures. Plasma was separated immediately and stored at −20°C until analysed for glucose (13), insulin (11) and ICSA.

ICSA were measured by modification of the 125I-protein A radioligand assay (4, 14), using an insulin-producing cell line, RINm5F, developed from a transplantable rat islet cell tumour (15). The cell line was kindly donated by Professor C. Hellerström, University of Uppsala, Sweden. The RINm5F cells were maintained in monolayer culture in medium RPMI 1640 containing 10% fetal calf serum at 37°C in an atmosphere of 5% CO2. Confluent cell cultures were rinsed in Ca++-free and Mg++-free Hanks Balanced Salt Solution (Ca/Mg-free HBSS) and incubated for 10 min at 37°C in Ca/Mg free HBSS containing 1 mM EDTA and 5 mg/ml bovine serum albumin (BSA). Cells were harvested and washed twice by centrifugation (100 g for 5 min) in Ca/Mg free HBSS and 5 mg/ml BSA. The dissociated cells were washed once in RPMI 1640 containing 5 mg/ml BSA at 4°C, and the number of cells was determined using a haemocytometer. The cells were used immediately for determination of ICSA. Plasma samples were heat-inactivated (30 min at 56°C) and diluted 1:10 in RPMI 1640 containing 5 mg/ml BSA. A 100 µl aliquot of suspended RINm5F cells (10⁵ cells/ml) was incubated with a sample of plasma (1:20 final plasma dilution) for 1 h at 4°C. Cells were washed twice by centrifugation (200 g for 2 min) in RPMI 1640 with 5 mg/ml BSA. The cell pellet was resuspended in 100 µl of RPMI 1640 with 5 mg/ml BSA and 125I-protein A (2.5 × 10⁶ c.p.m./ml) (Amersham International, Amersham, England). After incubation for 30 min at 4°C, the cells were washed three times by centrifugation in RPMI 1640 with 5 mg/ml BSA, and the cell pellet was counted in a gamma counter to determine cell-bound radioactivity. Blank incubations were performed as described above but with omission of plasma from the samples. Samples were considered ICSA positive when 125I-protein A binding exceeded 2 standard deviations above the mean binding value (4) of the 54 normal mouse plasma samples. The intra- and inter-assay coefficients of variation for the assay system were 6% and 14% respectively (n = 8), with non-specific binding (no plasma contributing less than 0.02% of total counts. The specificity of the 125I-protein A binding assay has been described elsewhere (4, 14).

Groups of data were compared using Student's unpaired t-test. Values were considered to be significantly different if P < 0.05.

Results

Aston ob/ob mice displayed characteristic age-related changes in body weight, plasma glucose and plasma insulin concentrations (Fig. 1). The
mice were obese (as indicated by increased body weight) and hyperinsulinemic at 4 weeks of age, with the onset of hyperglycaemia by 7 weeks. Moderate hyperglycaemia was maintained thereafter, despite markedly raised insulin concentrations which peaked at 10–15 weeks of age.

As shown in Fig. 2, the binding of $^{125}$I-protein A to RINm5F cells in the 54 normal control (+/+) mice ranged between 858 and 5803 c.p.m./$10^5$ cells ($2502 \pm 1196$, mean $\pm$ S.D.). In the present report a binding value exceeding the mean + 2 S.D. (i.e. $> 4894$ c.p.m./$10^5$ RINm5F cells) was considered to be ICSA positive (4). According to this criterion, 3 out of 54 (6%) normal +/+ mice and 3 out of 54 (6%) ob/ob mice were ICSA positive. ICSA positive plasma was not observed in ob/ob mice before the onset of diabetes, but was detected at 9 weeks in 1 out of 6, and in older mice at 20 weeks (1 out of 6) and 40 weeks (1 out of 3). At 20 weeks of age $^{125}$I-protein A binding produced by ob/ob plasma was greater ($P < 0.05$) than normal +/+ plasma. In contrast to ob/ob mice, ICSA positive plasma was only
Measurement of circulating islet cell surface antibodies (ICSA) by $^{125}$I-protein A binding to RINm5F cells after incubation with plasma from Aston obese hyperglycaemic (ob/ob) mice (■) and normal control (+/+) mice (□) between 4 and 40 weeks of age. Mean values are denoted by horizontal bars. The broken line shows the mean ± 2 S.D. of all values in +/+ mice. Values above this line were considered to be ICSA positive.

Discussion

Recent studies in man and experimental animals have suggested that autoimmune phenomena play a role in the pathogenesis of spontaneous diabetes (7). Thus, in addition to insulitis, lymphocytopenia and other indications of altered cell-mediated immunity, the diabetic condition is often associated with antibodies to cytoplasmic and cell surface antigens of islet B-cells. Although islet cytoplasmic antibodies are likely to be a consequence of islet B-cell damage, ICSA have been shown to mediate a complement-dependent cytotoxicity against islet B-cells (7–10). Abnormalities of cell-mediated immunity have been observed in ob/ob mice (16), but the possible involvement of autoimmune factors in the pathogenesis of this syndrome has not been considered. In the present study a $^{125}$I-protein A binding assay utilizing the RINm5F insulin-producing B-cell line was used to measure ICSA in ob/ob mice and normal (+/++) mice between 4 and 40 weeks of age. $^{125}$I-protein A binds specifically to the Fc portion of most subclasses of immunoglobulin IgG, and although binding to rodent IgG is lower than other species including man (17, 18), bound protein A radioactivity to RIN cells can be used to quantitate ICSA in rats (4). Application of this method in the present study indicates that surface antigens on RINm5F cells react with ICSA in both rat and mouse plasma.

During the development of diabetes in Aston ob/ob mice there are profound changes in B-cell function and islet morphology. Functionally the
B-cells selectively lose their responsiveness to glucose between 5 and 10 weeks of age (19), although they retain responsiveness to amino acids and neuro-endocrine components of the entero-insular axis (20-22), resulting in overall hyperinsulinaemia (19). Morphologically there is a marked degranulation and hyperplasia of the islet B-cells, with the appearance of degenerative islet lesions starting from about 15 weeks of age (12). These lesions include vacuoles with a distinctly ductular structure, which enlarge and replace B-cells, producing considerable islet necrosis in older mice (12). Insulitis is observed only very rarely (unpublished). The lack of ICSA positive plasma in ob/ob mice during the early phase of B-cell dysfunction and diabetes suggests that ICSA are not important in the aetiology and early pathogenesis of the diabetic condition. Indeed, ICSA were detected in 8-9-week-old control homozygous +++ mice which do not carry the genetic predisposition for diabetes. The observation of ICSA positive plasma in older ob/ob mice (1 in 6 at 20 weeks and 1 in 3 at 40 weeks) might reflect the degenerative changes characteristic of this age range.

References


