*Rapid communication***Induction of class II MHC antigens in vitro on pancreatic B cells isolated from BB/E rats**R. Walker¹, A. Cooke², A.J. Bone¹, B.M. Dean^{2,3}, P. van der Meide⁴ and J.D. Baird¹¹ Metabolic Unit, University Department of Medicine, Western General Hospital, Edinburgh,² Department of Immunology, The Middlesex Hospital Medical School, and³ Department of Diabetes and Immunogenetics, St. Bartholomew's Hospital, London, UK;⁴ The Primate Centre, Rijswijk, The Netherlands

Summary. The ability of recombinant Interferon- γ to induce class II expression in vitro on pancreatic islet B cells has been investigated by exposing islets isolated from BB/E and normal Wistar rats to Interferon- γ and then staining successively with monoclonal antibodies specific for rat class II MHC antigens and insulin. Induction of class II expression was never observed on islet cells obtained from either normal Wistar rats

or rats from the BB/E low diabetes incidence (<2%) subline. In contrast, pancreatic B cells from rats from the BB/E high diabetes incidence (60–70%) subline expressed class II antigen following culture with Interferon- γ .

Key words: BB rat, diabetes, Interferon- γ , pancreatic B cell, MHC class II expression.

We have previously shown that aberrant expression of class II MHC antigens occurs on pancreatic B cells in the spontaneously diabetic, insulin-dependent BB/Edinburgh (BB/E) rat [1]; this phenomenon has been described also in a case report of human Type 1 (insulin-dependent) diabetes mellitus [2]. Neither the significance of this finding nor the mechanism by which it occurs is understood. Recombinant Interferon- γ (r IFN- γ) has been shown to induce class II MHC antigens on both rat (DC Rayner, HJ de Assis-Paiva, PM Lydyard, S Bidey, P van der Meide, A Cooke, unpublished observations) and human [3] thyroid epithelial cells in vitro but not on normal rodent [4] or human [5] pancreatic B cells. Susceptibility to autoimmune disease in both man and BB rats [6] is known to be linked to the MHC complex. We have therefore examined the ability of rat r IFN- γ to induce class II antigens on pancreatic B cells isolated from normal Wistar and BB/E rats.

Materials and methods*Animals*

The animals used in this study were from the Edinburgh colony of BB rats, the nucleus of which was kindly donated by Dr. P. Thibert, Animal Resources Division of Canada, Ottawa in 1982. The colony now consists of two sublines created by selective breeding. In the high diabetes incidence line (BB/E/H) the incidence of insulin-dependent diabetes is 60–70% and the mean age at onset 96 days. In the low diabetes incidence line (BB/E/L) <2% of animals become diabetic by

120 days of age. BB/E/H animals were screened for circulating islet cell surface antibodies (ICSA) using a modified ¹²⁵I-protein A radio-ligand assay [7]. We have previously observed that over 90% of ICSA positive animals subsequently develop insulin-dependent diabetes (AJ Bone, unpublished observations). However, some ICSA negative animals also become overtly diabetic and require treatment with insulin.

Islet isolation, culture and dispersion

Islets were isolated by collagenase (Serva, Uniscience, London, UK) digestion from the following groups of animals, all of which were aged 80–90 days: 1) ICSA positive BB/E/H rats; 2) ICSA negative BB/E/H rats; 3) BB/E/L rats; 4) normal Wistar rats. For each experiment isolated islets were pooled from four rats in each animal group. Batches of pooled islets (350–400) were maintained in free-floating culture for 4–5 days in tissue culture medium (RPM1 1640, Imperial Laboratories, Salisbury, UK) containing glucose (11.1 mmol/l), HEPES (20 mmol/l), antibiotics (penicillin 100 U/ml; streptomycin 0.1 mg/ml), heat-inactivated 10% fetal bovine serum (Imperial Laboratories) with or without rat r IFN- γ (100 U/ml), a concentration we have previously established as optimal for Ia induction on cultured rat thyroid cells (DC Rayner et al., unpublished). The medium was changed after 2 days of culture. At the end of the culture period (4–5 days) islets were washed twice in ice-cold Ca²⁺ and Mg²⁺-free Hanks' Balanced Salt Solution containing penicillin and streptomycin (as above) and BSA (5 mg/ml) (HBSS-Ca²⁺/Mg²⁺). To prepare cell suspensions the washed islets were resuspended in HBSS-Ca²⁺/Mg²⁺ (1.5 ml) containing EGTA (2 mmol/l, Sigma Poole, Dorset, UK) and incubated for 20 min at 37°C in a shaking water bath to disperse the islet cells. Cell dispersion was completed by repeated (10 times) aspiration in a Pasteur pipette. The cell suspension was washed once in HBSS-Ca²⁺/Mg²⁺, once in RPM1 1640 (supplemented as above) and finally resuspended in RPM1 1640 (approximately 5 × 10⁵ cells/ml) for staining.

Fig. 1 a and b. Cell suspension (primary magnification $\times 400$) from BB/E/H rats cultured with r IFN- γ and stained by immunofluorescence using monoclonal antibodies. **a** Expression on all 3 cells in this field; **b** Double exposure photograph taken successively using normal green and red filters on a Leitz fluorescence microscope showing that the 3 class II positive cells contain insulin.

Islet cell immunofluorescence staining

The following monoclonal antibodies were generously provided by Dr. D. Mason, Sir William Dunn School of Pathology, Oxford: MRC OX-6, which recognises a non-polymorphic determinant on rat Ia, MHC Class II antigen; MRC OX-8, which recognises T cytotoxic lymphocytes and was used as a control for non-specific binding. Clone supernatants were used at 1:5 dilution in PBS (0.05 mol/l pH 7.6) containing 10% normal rat serum and L-lysine (20 mmol/l, Sigma). Islet cells (2×10^4) were incubated in the monoclonal antibody (100 μ l) at 0–4 °C for 1 h. Following washing in PBS (0.05 mol/l, pH 7.6) containing L-lysine, (20 mmol/l) surface staining was revealed after a similar incubation with rhodaminated rabbit anti-mouse immunoglobulin (Dako Ltd., High Wycombe, Bucks, UK) previously adsorbed with rat liver acetone powder (Sigma). After washing, surface-stained cells were fixed in ice-cold 2% paraformaldehyde in 0.9% NaCl containing HEPES (20 mmol/l) and BSA, (1 mg/ml) pH 7.4 for 30 min. The cell suspensions were washed and put on to glass slides, air-dried and treated with 50% ethanol for 15 min at –20 °C and washed again in PBS. Slide preparations were incubated for 40 min at 25 °C with clone supernatants of monoclonal anti-insulin (2 FI), anti-glucagon (GLU 001) or anti-somatostatin (SOM 018) antibodies (kindly donated by Novo, Copenhagen, Denmark). Reaction was revealed using biotinylated horse anti-mouse immunoglobulins (Sera-Lab, Crawley Down, Sussex, UK) followed by fluoresceinated streptavidin (Cambridge Bioscience, Cambridge, UK). Slides were examined under a fluorescence microscope (Leitz, Luton, UK) equipped for double fluorochrome studies. Photographs were taken on Ektachrome ASA 400 film with automatic exposure.

Experimental protocol

Batches of pooled islets obtained from 4 animals on each occasion were isolated, cultured and stained from: 1) ICSA positive BB/E/H rats on 5 separate occasions ($n = 20$); 2) ICSA negative BB/E/H rats on 4 separate occasions ($n = 16$); 3) BB/E/L rats on 4 separate occasions ($n = 16$); and 4) normal Wistar rats on 3 separate occasions ($n = 12$).

Results and discussion

In agreement with other groups [4], induction of class II MHC antigen by r IFN- γ was never observed on any islet cells obtained from normal Wistar rats. Additionally, we found that r IFN- γ failed to induce expression of class II antigen on the surfaces of insulin containing B cells from BB/E/L animals. In contrast, islet B cells from BB/E/H animals, whether ICSA positive or negative, expressed class II antigen following culture with the same dose of r IFN- γ (Fig. 1). In agreement with our previous findings [1] class II expression was not induced on glucagon or somatostatin secreting islet cells from BB/E/H animals.

In our experiments 80% of the endocrine cells in any field were B cells, and on average 50% of these were induced to express class II antigen. Incubation of islet cells with the irrelevant monoclonal antibody MRC OX-8 (as a check for non-specific binding) produced no cell surface staining. OX-6 positive non-endocrine cells were seen only occasionally in any of the cell suspensions, which was not unexpected, since maintenance of isolated islets in free-floating culture for several days has been shown to act as a purification step for removal of non-endocrine cells [8].

Since expression of class II MHC antigen was not observed on any islet cells cultured in the absence of r IFN- γ , the expression of Ia antigen on B cells from BB/E/H animals is unlikely to be due to adsorption of shed Ia from passenger macrophages. These findings therefore suggest that the ability of r IFN- γ to induce class II expression may depend on genotype. Genetic factors could influence islet cell susceptibility to r IFN- γ

by several possible mechanisms. Islets obtained from diabetes prone (BB/E/H) animals may have been damaged already; for example, by cytokines such as Tumour Necrosis Factor or Interleukin 1 produced by activated macrophages. The presence or absence of ICSA does not appear to be related to the sensitivity of B cells to r IFN- γ . However, since circulating ICSA levels fluctuate in the pre-diabetic period it is likely that some animals categorised as ICSA negative had been ICSA positive at some time previously.

It has been suggested that aberrant expression of class II antigen on endocrine cells may be an initiating feature of autoimmune disease [2, 9]. However, our previous studies in the BB/E rat led us to postulate that expression of class II antigen on pancreatic B cells was a consequence rather than a cause of pancreatic mononuclear cell infiltration [1]. Since IFN- γ is produced only by T cells, our present findings add further weight to our concept of the sequence of events leading to the development of overt insulin-dependent diabetes.

Acknowledgements. This work was supported by grants from the Medical Research Council (UK), the Scottish Hospital Endowments Research Trust, The Wellcome Trust and The British Diabetic Association. We would like to thank Mr. D. Brown and W. Smith for their skill in caring for the animals involved in these studies. We are also grateful to Professor I. M. Roitt for his support and encouragement.

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Received: 29 July 1986
and in revised form: 1 September 1986

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