Reactivity of Pancreas Islet Cells With Antisera of Known Specificity

K. Ulrichs, T. Schang, R. Keller, and W. Müller-Ruchholtz

IN NON-MHC (major histocompatibility complex) as well as in MHC fully allogeneic rat strain combinations, islet grafts are very often as rapidly rejected as skin grafts. Among the many attempts to reduce islet immunogenicity, there are promising studies such as the in vitro culture of islet grafts prior to transplantation,[1] namely, the elimination of Ia-bearing cells from murine islets with antibody directed against Ia determinants.[2] Little is known about the cell type(s) and number of immunogenic cells in question. Therefore, it was the aim of this study to (1) characterize rat islet cells with sera of known specificity, (2) try to eliminate putatively immunogenic cells serologically without damaging the insulin-secreting cells, and (3) test the in vivo relevance of the in vitro observations.

MATERIALS AND METHODS

Animals

Pancreas islet and cell donors were male CAP ($RT1^c$) rats. Graft recipients were LEW ($RT1^l$) rats, made diabetic by a single intravenous (IV) injection of 55 mg/kg streptozotocin (Upjohn, Kalamazoo, Mich). Animals were bred and housed at the Department of Immunology.

Islet Isolation

Approximately 1,500 handpicked clean islets were isolated from ten donor animals using the collagenase technique.[3]

Islet Dispersion

Fresh islets were enzymatically dispersed into a single-cell suspension with dispase I enzyme (Boehringer, Mannheim, FRG) in three to six 15-minute steps (each step with 4 mL Ca^{2+}-free Hepes-buffered HBSS, pH 7.5, 37°C, gentle shaking) using a total of 10,000 IU. The supernatants containing single cells were washed twice and collected. The total cell number varied between 10^5 and 10^6 cells per dispersion, depending on the number of islets and the enzyme activity.

Tests

Standard immunofluorescence tests were performed in Terasaki microplates (Greiner, Nürtingen, FRG) with either rat lymph node cells, thymocytes, splenic macrophages, or dendritic reticulum cells from the spleen as control cells, and dispersed pancreas islet cells as test cells. Findings were evaluated comparing fluorescence and phase contrast appearance of cells. Standard cytotoxicity tests were carried out with the above cells in Cooke microtiter plates using fresh or frozen LEW complement. Standard immunoperoxidase tests were performed with total fresh islets on microscopic slides.

Test Sera

Monoclonal antibody (rat to rat) YR5/24 against class II antigens (RT1B region) was commercially obtained (Serotec, Bicester, UK) and used in 1:100 dilutions in the above tests. Monoclonal antibody (mouse to rat) 0X6 against class II antigens (RT1B region) was a gift from Dr Wonigeit (Hannover, FRG) and used in 1:200 dilutions in the above tests. Rabbit-anti-rat lymphocyte serum (ALS) was prepared, absorbed, and tested as described in detail elsewhere.[4] To prepare specified anti-lymphocyte serum (SAL), sequential absorptions with erythrocytes (E), peritoneal exudate cells (P), and fetal liver cells (Lf) were performed. It may be pointed out that each absorption was proved to be serologically exhaustive, maintaining 100% cytotoxicity at dilutions of 1:4 to 1:16 in sera that were only used undiluted. Rabbit-anti-rat macrophage serum (AMS) was prepared, absorbed, and tested similar to ALS, using peritoneal exudate cells (10^8) for immunization. To prepare specified antimacrophage serum (SAM), sequential absorptions with erythrocytes, thymocytes (T), lymphocytes (L), and adult liver cells (La) were performed.

RESULTS

Reactivity With Monoclonal Anti-Ia Antibodies

As shown in the first row of Fig 1, among the freshly suspended islet cells containing

From the Departments of Immunology and General Surgery, University of Kiel, Kiel, FRG.

Address reprint requests to K. Ulrichs, Department of Immunology, University of Kiel, Brunswiker Str 2–6, D–2300 Kiel 1, FRG.

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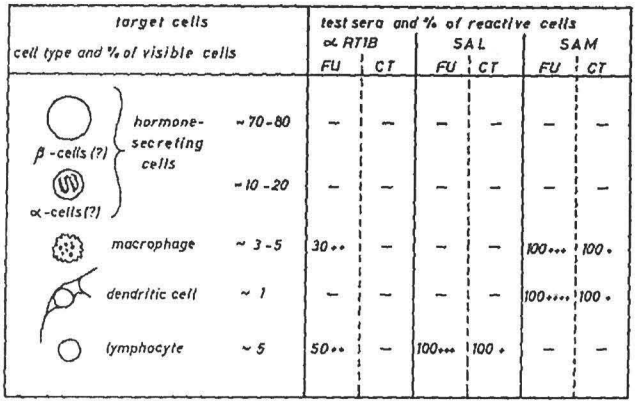

Fig. 1. Pancreas islet cell reactivity in fluorescence (FU) and cytotoxicity (CT).

mainly hormone-secreting cells, 30% of the visible macrophages, 3% to 5% of the total cell number, and 50% of the visible lymphocytes, approximately 5% of the total cell number, reacted positively with YR5/24. However, as also shown, islet dendritic cells (I-DC, approximately 1% of the total cell number) did not react with anti-Ia in contrast to the known reactivity of spleen dendritic control cells. (The antibody reacted clearly positive with 30% to 40% of the control lymphocytes and 50% of the control macrophages, thus confirming the suppliers' data; data not shown).

As seen in Fig 1, the antibody was nontoxic to dispersed islet cells, when tested in combination with rat complement in cytotoxicity assays. These and the above results have been confirmed using the second monoclonal anti-Ia antibody 0X6.

Reactivity With SAL

Exhaustive absorptions with erythrocytes and peritoneal exudate cells yield an antiserum (ALS-EP) that specifically reacts with control lymphocytes and thymocytes (data not shown). After elimination of the critical crossreactivity of ALS-EP with the hormone-secreting islet cells by additional absorptions with adult or fetal liver cells, approximately 5% lymphocytes could be detected in the islet cell suspension (see Fig 1). Furthermore, SAL proved to be 100% cytotoxic when used in combination with rat complement.

Reactivity With SAM

Exhaustive absorptions with erythrocytes, thymocytes, and lymphocytes yield an antiserum (AMS-ETL) that specifically reacts with control macrophages and dendritic reticulum cells of the spleen (data not shown). After elimination of the critical crossreactivity of AMS-ETL with the hormone-secreting islet cells by additional absorptions with adult or fetal liver cells, 3% to 5% macrophages and approximately 1% of cells showing a characteristic dendritic structure (both of which reacted strongly with SAM) could be detected in the suspension (see Fig 1). Similar to SAL, SAM was 100% cytotoxic when used in combination with rat complement.

Characterization of an Islet Dendritic Cell

In fresh single-cell suspensions I-DCs are only observed when islet dispersion is performed with dispase of very low activity. Their serologic pattern is Ia^-, SAL^-, and SAM^{++++}, and they are easily lysable in cytotoxicity tests. They are of typical dendritic appearance, as can be seen from single cells in immunofluorescense as well as phase contrast (data not shown) and from total islets in immunoperoxidase tests (see Fig 2). Morphologically, they are often irregularly shaped cells frequently with very long plasmatic extensions. They develop only one to two hours after preparation of cell suspension. I-DCs never stain in background fluorescence or in immunoperoxidase control tests using normal rabbit serum as first antibody.

In Vivo Data After SAL and SAM Incubation of Islet Grafts: A Study in Progress

A preliminary finding in a MHC fully and biologically strong histoincompatible rat strain combination ($RT1^c \rightarrow RT1^l$ in which grafts are always rejected between five and six days) suggests that fresh islets pretreated with SAL plus SAM and complement are accepted in the long run (>50 days) without any immunosuppression of the graft recipient, provided

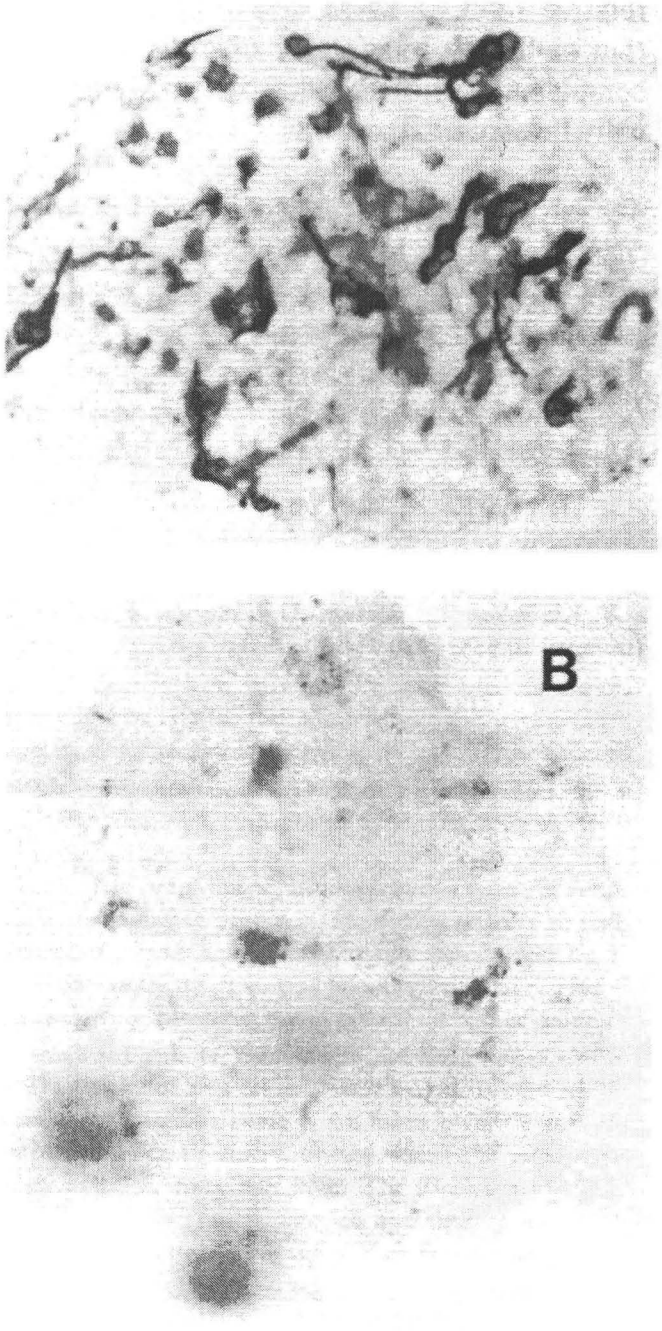

Fig 2. Immunoperoxidase test with freshly isolated pancreatic rat islets. (A) SAM-positive dendritic cells within the islet. (B) No staining with normal rabbit serum as first antibody. ×250.

the antiserum treatment is performed with sufficiently large amounts of antibodies over a sufficiently long period of time.

DISCUSSION

Immunofluorescence tests and phase contrast microscopy with dispase-dispersed rat islet cells show that clean handpicked islets contain a distinct number of lymphocytes and macrophages, each of which contain Ia^+ cells. The existence of such cells within the islet of Langerhans has previously been suggested,[5] however, precise data have not been published so far.

An unexpected result obtained from our studies is the identification of an islet dendritic cell which appears to be Ia^-, SAL^-, and SAM^{++++}. This is in contrast to the observation of Hart and Fabre,[6] who identified Ia^+ interstitial dendritic cells in rat islet frozen tissue sections. (The serologically and morphologically detailed characterization of isolated cells will be of greater value for further evaluation of the I-DC as an immunogenic cell than histologic studies). The Ia negativity of a cell with obvious dendritic morphology is a striking phenomenon, as it is generally believed that this cell serves for antigen presentation requiring the expression of class II antigens. This may be a result of the methodologic procedure having caused a damaging effect on the Ia determinant, as the cells were studied within only few hours after enzymatic dispersion. In vitro culture prior to immunofluorescence may help to restore antigen expression (study in progress).

On the other hand, lymphocytes as well as macrophages clearly contain Ia^+ cells after the serum incubation procedure, which suggests that I-DCs are at least much more labile Ia-antigenic determinant expressors than those cells. This may be seen in context with increasing evidence from the recent literature that Ia expression on certain cell surfaces may be variable, dependent on immunologic[7] or nonimmunologic[8] stimuli. From this labile expression of Ia, it could be inferred that the approach of islet incubation in SAM (plus SAL), ie, with cell group-specific antibodies, may well prove to be superior for allogeneic islet grafting.

The I-DCs clearly differ from islet fibroblasts, which are also Ia^-, but are numerous, have a typical spindlelike morphology, and react only weakly with SAM. Pretreatment of mouse islet allografts with Ia antibodies has been shown to be effective in an MHC-histoincompatible model[2] which, however,

appears to have been relatively weak in biologic terms (as inferred from control graft survival data). In contrast, our rat islet allograft model appears to be much stronger (consistent control graft rejection time, 5–6 days). It is hoped that the preliminary findings obtained in this model and use of SAL and SAM for islet incubation can be extended and further analyzed (with regard to the relative roles of SAM and SAL), provided that sufficient penetration of antibodies and complement into the islet can methodologically be assured.

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