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HLA Incompatibility and Immunogenicity of Human Pancreatic Islet Preparations Cocultured with Blood Cells of Healthy Donors

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ABSTRACT: Type 1 diabetes mellitus (T1D) is a T-cell–mediated autoimmune disease characterized by the destruction of beta cells in the pancreas. An attractive novel therapy for type 1 diabetes is pancreatic islet transplantation, provided that recurrent islet autoimmunity and allograft rejection can be prevented. We analyzed the response of peripheral blood mononuclear cells (PBMC) from healthy blood donors to human islet-cell preparations with a composition similar to that of islet grafts used in clinical transplantation trials. It was examined whether the degree of major histocompatibility complex incompatibility between PBMC and donor islet cells is related to the degree of proliferative T-cell responses during coculture of human leukocyte antigen (HLA)–matched and mismatched PBMC with human islet cell-preparations (*i.e.,* mixed islet/lymphocyte reaction). Prominent T-cell responses were observed in the vast majority of cases of double HLA class II mismatches. Intermediate T-cell responsiveness was observed in single HLA class II mismatches, whereas HLA matches did not induce a T-cell response. Our results identify the potential immunogenicity of islet preparations transplanted between HLA-DR incompatible subjects regardless of an autoimmune background of the recipient. *Human Immunology 66, 494–500 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: type 1 diabetes; islet transplantation; alloreactivity; HLA; mixed islet/lymphocyte reaction

ABBREVIATIONS

MILR	mixed islet/lymphocyte reaction
MLR	mixed lymphocyte reaction
PBMC	peripheral blood mononuclear cells
T1D	type 1 diabetes mellitus

INTRODUCTION

Because the major histocompatibility complex (MHC) molecule plays an essential role in the activation of T-cell responses, the genes encoding these molecules have been implicated in susceptibility to serve T-cell–mediated autoimmune diseases. Several human leukocyte antigen (HLA) alleles have been demonstrated to be major genetic risk factors in development of type 1 (insulin dependent) diabetes mellitus (T1D). Vulnerability for T1D is genetically dominated by the HLA gene region, accounting for 42% of the familial inheritance of T1D [1].

T1D is an autoimmune disease characterized by the specific destruction of beta cells in the pancreas. The etiology of T1D is multifactorial, consisting of genetic predisposition and environmental factors, including a variety of viruses and dietary components [2]. It has long been acknowledged that T cells play a crucial role in the immunopathogenesis of T1D, the hallmark of autoimmune diabetes being lymphocytic invasion of pancreatic islets [3–10].

A potential therapy for diabetes is transplantation of insulin-producing beta cells of isolated pancreatic islets, provided that recurrence of T-cell autoreactivity against islet determinants and induction of alloimmunity to

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donor antigens are prevented [11–13]. This attractive and recently successful therapy for T1D is overshadowed by the need for permanent immune suppression. Without the administration of these nonspecific and potentially harmful immunosuppressive drugs, graft failure seems inevitable. Islet transplantation is thus limited to patients with diabetes already receiving immune suppression for a previous organ transplant, or to patients with severe hypoglycemia unawareness or uncontrollable hyperglycemia. The introduction of a new glucocorticoid-free immunosuppressive regime, the so-called Edmonton protocol, has considerably improved the outcome of islet transplantation [14]. This protocol includes sirolimus, tacrolimus, and daclizumab. All these immunosuppressive drugs share the same basic quality: they all inhibit T-cell stimulation and proliferation, a finding that identifies T cells as key players in this rejection process.

Prediction and prevention of ongoing beta-cell destruction after islet transplantation resulting in long-term graft survival is of utmost importance. In order to be able to optimize the current islet transplantation, it is essential to study the reaction of T cells to islets.

In rat studies, it has been demonstrated that T cells obtained before islet transplantation can react against islet allografts in a mixed islet/leukocyte culture [15]. This reactivity was similar to that of T cells isolated after grafting. We have previously demonstrated a marked increase in T-cell alloreactivity in immunosuppressed T1D patients rapidly on implantation of human islet allografts from multiple donors [11]. This increase was exclusive to patients who experienced acute rejection of the islet allograft; it was absent in cases of successful restoration of beta-cell function. The contribution of HLA class II to immunogenicity of islets was further underscored by our recent observation that islet autoantigens are processed and presented by vascular endothelial cells expressing MHC class II leading to activation of autoreactive T cells [16], implying that MHC class II could be important in human islet graft failure by autoreactivity [17]. Little is known about the extent that human islet preparations could be target of alloreactivity in relation with the degree of HLA mismatching with human healthy blood donors.

To evaluate the potential immunogenicity of human islet preparations under immunocompetent conditions, we investigated the ability of peripheral blood mononuclear cells (PBMC), isolated from immunologically uncompromised healthy blood donors, to react human islet preparations in relation with the degree of HLA-DR incompatibility. Mixed islet/lymphocyte cultures [18, 19] were performed with a panel of HLA-DR–matched and –mismatched healthy blood donors. For comparison, mixed lymphocyte reactions (MLR) that used PBMC of blood donors with PBMC of the islet donor were carried out and analyzed in relationship to the pattern of reactivity found in the mixed islet/lymphocyte reaction (MILR).

MATERIALS AND METHODS

Human Islet Isolation and Culture

Human pancreata were obtained from organ donors (11 preparations from 10 donors, aged 2–60 years) at European hospitals affiliated with β-Cell Transplant, a European Concerted Action on islet-cell transplantation in diabetes. Islets were prepared in the central unit of this multicenter program (Medical Campus, Vrije Universiteit Brussels, Brussels, Belgium) [12]. Detailed descriptions of methods of isolation, and phenotypic and functional characterization of the islet preparations are available elsewhere [12]. This study was conducted with cultured islet-cell preparations comparable to those incorporated in grafts that are implanted in patients. They are composed of endocrine and nonendocrine duct cells with, in average, 48% insulin-positive cells (Table 1). Their content in MHC class II–expressing cells is consistently lower than 1% (Figure 1). The available beta-cell mass was too low for inclusion in grafts so that the preparations became available for approved research projects if they fulfilled the legal and ethical criteria that were set for such use. The isolated islet preparations were cultured in Ham F10 medium [12] for 1–5 days before transfer to Leiden, where they were immediately processed for immunological studies.

PBMC Isolation

PBMC were obtained from healthy blood donors visiting the blood bank in Leiden, the Netherlands, by Ficoll separation after obtaining the donors' informed consent.

HLA-DR Typing

The HLA-DR genotypes of both blood and pancreas donors were determined by conventional genotyping in most cases, as described in detail elsewhere [20]. Pancreata were HLA typed by serology only. Therefore, all HLA genotypes were translated to serotype to allow comparison.

MILR

Islet preparations in a concentration range (10,000 to 150,000 endocrine cells per well) were cocultured with 150,000 PBMC per well in microwell tissue plates for 5 days at 37°C, 5% carbon dioxide in air. Islet preparations and PBMC were HLA-DR matched or mismatched. RPMI 1420 culture medium was used containing 10% fetal calf serum and 100 units/ml penicillin and 100 μg/ml streptomycin. Samples were incubated in triplicate. On day 5, [H^3]-thymidine was added to each well for 16 hours in an end concentration of 1 μCi/ml. The