Innate and adaptive immunity to human beta cell lines: implications for beta cell therapy

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Abstract
Aims/hypothesis Genetically engineered human beta cell lines provide a novel source of human beta cells to study metabolism, pharmacology and beta cell replacement therapy. Since the immune system is essentially involved in beta cell destruction in type 1 diabetes and after beta cell transplantation, we investigated the interaction of human beta cell lines with the immune system to resolve their potential for immune intervention protocol studies.
Methods Human pancreatic beta cell lines (EndoC-βH1 and ECi50) generated by targeted oncogenesis in fetal pancreas were assessed for viability after innate and adaptive immune challenges. Beta cell lines were pre-conditioned with T helper type 1 (Th1) cytokines or high glucose to mimic inflammatory and hyperglycaemia-stressed conditions. Beta cells were then co-cultured with auto- and alloreactive cytotoxic T cells (CTL), natural killer (NK) cells, supernatant fraction from activated autoreactive Th1 cells, or alloantibodies in the presence of complement or effector cells.
Results Low HLA expression protected human beta cell lines from adaptive immune destruction, but it was associated with direct killing by activated NK cells. Autoreactive Th1 cell inflammation, rather than glucose stress, induced increased beta cell apoptosis and upregulation of HLA, increasing beta cell vulnerability to killing by auto- and alloreactive CTL and alloreactive antibodies.
Conclusions/interpretation We demonstrate that genetically engineered human beta cell lines can be used in vitro to assess diverse immune responses that may be involved in the pathogenesis of type 1 diabetes in humans and beta cell transplantation, enabling preclinical evaluation of novel immune intervention strategies protecting beta cells from immune destruction.

Keywords Adaptive immunity · Beta cell · Innate immunity · Transplantation

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Abbreviations
B-LCL B-lymphoblastoid cell lines
CMV Cytomegalovirus
CTL Cytotoxic T cells
EF1α Elongation factor 1-alpha
MFI Mean fluorescence intensity
MSC Mesenchymal stromal cell
NK Natural killer
PBL Peripheral blood lymphocytes
PBMC Peripheral blood mononuclear cells
PPI Preproinsulin
PTEC Primary tubular epithelial cell
Th1 T helper type 1
Introduction

Beta cell replacement by pancreas or islet transplantation is currently the only curative treatment for established type 1 diabetes. Insulin independence using current islet transplantation protocols is often temporary despite aggressive immune suppression. Both innate and adaptive immune responses threaten transplanted beta cells and need to be controlled by immune suppression [1–3]. More effective and less toxic strategies are required to make beta cell transplantation affordable to more patients.

Knowledge of interactions of human beta cells with the immune system has been largely derived from studies on isolated islets from pancreas donors. Access to such preparations for scientific purposes is limited; furthermore, variations between islet preparations and their composition, including a range of other cell types, hinder beta cell-specific studies. Human genetically engineered beta cell lines provide a novel tool to study functional human beta cells in standardised assays [4]. Thus, beta cell lines may help to identify immune responses relevant to human type 1 diabetes and beta cell transplantation.

We investigated innate and adaptive immune responses potentially harmful to beta cells in the pathogenesis of type 1 diabetes and beta cell transplantation on genetically engineered human beta cell lines to assess their potential for preclinical evaluation of novel immune intervention strategies.

Methods

Two human fetal beta cell lines with similar function (EndoC-βH1 and ECi50; Endocells, Paris, France) were generated and maintained as previously reported [4]. To mimic inflammation or hyperglycaemia, beta cell lines were preincubated overnight with IFNγ (1,000 U/ml; R&D Systems, Abingdon, UK) or glucose 20 mmol/l. Introduction of EF1α promoter-driven HLA-A*02:01 into beta cell line EndoC-βH1 was achieved by lentiviral transduction [5]. HLA genotyping was carried out at the Eurotransplant Reference Laboratory, Leiden University Medical Center, Leiden, the Netherlands, cultured and activated with IL-15 as described [6]. Details about generation and maintenance of specific T cell clones, immortalised human primary tubular epithelial cells (PTEC), HeLa, Epstein–Barr virus-transformed B lymphocytes, mesenchymal stromal cells (MSC) and human monoclonal antibodies recognising HLA have been previously published [7–11].

Beta cell-specific T helper (Th) cell supernatant fraction was harvested from 3 day cultures of autoreactive Th1 clone 1c6 incubated with PBMC and preincubated with or without antigen [12]. Supernatant fraction was stored at −80°C until use.

Cellular cytotoxicity was assessed by chromium release of 51Cr-labelled beta cell lines. Complement-dependent cytotoxicity was measured by flow cytometry of beta cell lines after incubation with human HLA-specific antibodies and rabbit complement. Cytokine-driven beta cell death was measured by propidium iodide staining and flow cytometry after 48 h culture in Th1 cell supernatant fraction or 50 U/ml IL-1β, 1,000 U/ml IFNγ and 1,000 U/ml TNF-supplemented medium. Cell surface antigen expression was assessed by flow cytometry.

Experiments were not blinded. Experiments were excluded if positive controls did not respond or with responding negative controls. Mycoplasma infection was excluded for all cell lines at regular intervals.

Data are represented as mean and SD unless stated otherwise. Statistics represent linear regression for titrated experiments and Student’s t test for binary outcomes. GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA) was used to create graphs and perform analysis. Further details are given in the electronic supplementary material (ESM methods).

Results

Cytokine-mediated effects on beta cells

Two human beta cell lines (EndoC-βH1 and ECi50) were selected for immunological analysis. Cells were genotyped as HLA-A*33:03, A*68:01 (EndoC-βH1) and HLA-A*02:02, A*68:01 (ECi50). HLA class I expression on EndoC-βH1 was slightly lower than on ECi50 (geo-mean 1,000 vs 1,11 [7]). Supernatant fraction was stored at −80°C until use.

Peripheral blood mononuclear cells (PBMC) were separated from full blood or buffy coats (for natural killer [NK] cells and lymphocytes) by Ficoll-Hypaque density gradient. Peripheral blood lymphocytes (PBL) were separated by CD14 depletion of PBMC with CD14 MicroBeads (Miltenyi Biotec, Auburn, CA, USA). NK cells were purified from PBMC using the human NK Cell Isolation Kit (Miltenyi Biotec, Leiden, the Netherlands), cultured and activated with IL-15 as described [6]. Details about generation and maintenance of specific T cell clones, immortalised human primary tubular epithelial cells (PTEC), HeLa, Epstein–Barr virus-transformed B lymphocytes, mesenchymal stromal cells (MSC) and human monoclonal antibodies recognising HLA have been previously published [7–11].

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Supernatant fraction of activated T cells increased HLA class I, but not class II, expression, similar to incubation with IFN-γ (Fig. 1b). Supernatant fraction of activated T cells increased beta cell death from 46±5% to 70±2% (p<0.0001; n=3) for EndoC-βH1, and from 36±6% to 59±5% (p<0.0001; n=3) for ECi50. Comparably, incubation with mixed cytokines (IFN-γ 1,000 U/ml, TNF 1,000 U/ml and IL-1β 50 U/ml) increased beta cell death from 22±6% to 40±8% (p=0.0003; n=4) for EndoC-βH1 and from 22±5% to 35±
alloreactive CTLs after HLA upregulation was verified by
CTLs (Fig. 1g). Specific recognition of beta cell lines by
glucose) preincubation did not affect killing by alloreactive
(4 h cytotoxicity assay only if HLA was upregulated by IFN
γ). The expression of the cytolytic degranulation marker CD107a on
responding CTLs (data not shown).

Cell-mediated cytotoxicity

Destruction of beta cells by autoreactive cytotoxic T cells
(CTL) is the hallmark of type 1 diabetes. We therefore inves-
tigated autoreactive preproinsulin (PPI)-specific CTL re-
sponses to endogenous expression of beta cell antigens by
the cell lines. Since our effector T cell clones are HLA-A2
(*02:01)-restricted and the beta cell lines were lacking HLA-
A2, expression had to be introduced. Beta cell line
EndoC-βH1 was transduced with HLA-A*02:01 under the
elongation factor 1-alpha (EF1α) promoter. After passaging,
the generated line contained 39% HLA-A2-positive cells and
was stable for at least 12 passages. Expression of transduced
HLA-A*02:01 was MFI 118 and was unaffected by IFNγ.

Overnight incubation of the HLA-A2-transduced beta cell
line with PPI-specific cytotoxic T cells resulted in beta cell
cytolysis up to 34±3% (p<0.0001 for intercept; n=4) without
adding exogenous PPI peptide epitope, corresponding to HLA-
A2 expressing cell frequency (Fig. 1h). Pulsing of the trans-
duced beta cell with exogenous cytomegalovirus (CMV) pep-
tide epitope (mimicking CMV infection) resulted in killing by
CMV-specific CTLs with similar efficacy (data not shown).

Alloreactive CTLs can cause beta cell allograft rejection
after transplantation. Thus, beta cells were tested against
HLA-A*02:02-specific alloreactive CTLs. A beta cell line nat-
urally expressing HLA-A*02:02 was killed (up to 66±5%) in a
4 h cytotoxicity assay only if HLA was upregulated by IFNγ
(p=0.005 for intercept; n=3). Hyperglycaemic (>25 mmol/l
glucose) preincubation did not affect killing by alloreactive
CTLs (Fig. 1g). Specific recognition of beta cell lines by
alloreactive CTLs after HLA upregulation was verified by
expression of the cytolytic degranulation marker CD107a on
responding CTLs (data not shown).

Low HLA expression by the beta cell lines may render
these cells susceptible to NK cell reactivity. Indeed, activ-
ated NK cells killed beta cell line EndoC-βH1, which
expresses relatively less HLA more efficiently than ECi50
(up to 47±4% and 28±0%, respectively; p=0.016 for
slope; n=2). HLA upregulation reduced killing to 38±
2% (p=0.002 for intercept) for EndoC-βH1 and 11±1%
(p=0.0003 for slope) for ECi50 (Fig. 1i). Hyperglycaemia
did not influence NK cell killing of beta cell lines. Results
were corroborated by a CD107a degranulation assay (data
not shown).

Antibody- and complement-mediated killing

Antibodies recognising HLA can lead to acute rejection of
transplants through activation of immune cells or comple-
ment. Low HLA expression protected from antibody-
dependent cellular cytotoxicity by PBL or purified NK
cells. Yet, HLA upregulation increased killing through
alloreactive antibodies (for EndoC-βH1 up to 38±7%
through NK cells [p=0.002 for intercept; Fig. 1j]) and
up to 49±6% through PBL [p<0.0001 for slope; Fig. 1k]). Complement inhibitory receptors generally pre-
vent direct complement activation, and beta cell lines
expressed CD59 and CD46, but not CD55 (Fig. 1d–f). Beta
cell lines were thereby protected from killing by human
serum complement.

To assess their killing potential, alloantibodies were titrated
in standard clinical cross-match assays using rabbit comple-
nent. Specific alloreactive antibodies induced >80%
complement-dependent cytotoxicity of beta cell lines upon
upregulation of HLA by IFNγ, whereas alloantibodies direct-
ed to HLA not expressed by the human beta cell lines had no
such effect (p=0.006 for slope) (Fig. 1l).

Table 1 Overview of results

| Interaction with immune system | Conditioning of beta-cell lines | Inflammatory cytokines | Glucose challenge |
|--------------------------------|--------------------------------|------------------------|------------------|
| HLA expression                 | Lower than other tissue cell lines | Increased | Unchanged (i.e. low) |
| Autoreactive Th cell supernatant| Moderate apoptosis | NA | Moderate apoptosis |
| Autoreactive CTL recognition and killing | Proinsulin-specific killing | NA | Proinsulin-specific killing |
| Alloreactive CTL recognition and killing | Immune response | Strong immune response | Immune response |
| NK cell recognition and killing | Recognition and differential killing | Decreased killing | Recognition and differential killing |
| ADCC                           | No killing with HLA antibodies | Concentration-dependent killing | ND |
| CDC                            | No killing with HLA antibodies | Concentration-dependent killing | ND |

ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; NA, not applicable; ND, no data

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Discussion

We investigated immune responses to human beta cell lines that may be relevant for diabetes pathogenesis and beta cell transplantation, demonstrating the relevance of these beta cell lines for preclinical studies on immune intervention strategies (Table 1).

Studies of type 1 diabetic pancreases suggest that autoreactive cytotoxic T cells are highly efficient killers of beta cells [13]. We confirm that autoreactive T cell clone 1E6 can efficiently kill the beta cell lines that were HLA compatible, which substantiates that these beta cell lines can process and present PPI15–24 epitope from endogenously produced PPI to the immune system. This establishes these cell lines as bona fide beta cells in terms of their susceptibility to diabeticogenic autoimmune reactions.

Alloreactive responses may be detrimental for transplanted beta cells too. We show that beta cell lines become sensitive to killing by donor-specific alloreactive CTLs or alloantibodies if HLA is upregulated by inflammation. At the same time, low HLA expression left unstimulated beta cell lines vulnerable to activated NK cells. These data support clinical observations that suppressing early inflammation may be as important for transplant success as immunosuppression targeting adaptive immunity. Whether normal human beta cells express equally low HLA remains unknown, since HLA expression by human beta cells purified from isolated islets is difficult to quantify. However, HLA class I is markedly upregulated in pathogenic conditions including insulitis in islets of type 1 diabetic patients [13]. We confirm that supernatant fraction of autoreactive T cells from a patient with type 1 diabetes responding to islet antigen can upregulate HLA on beta cell line cells. Moreover, these supernatant fractions increased beta cell death, similar to previously described inflammatory cytokines [2].

In conclusion, we demonstrate that genetically engineered human beta cell lines can be used in vitro to assess diverse immune responses that may be involved in the pathogenesis of type 1 diabetes in humans and in beta cell transplantation. This enables human preclinical evaluation of novel immune intervention strategies protecting beta cells.

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Duality of interest R. Scharffmann, P. Czernichow and P. Ravassard are shareholders and consultants for Endocells.

Contribution statement CRvdT designed and performed experiments and wrote the manuscript. AZ designed and performed transduction experiments and wrote the manuscript. DLR and SHB-S designed and performed antibody and complement experiments and revised the article. GD designed and performed cellular killing experiments and revised the article. MP provided the 1E6 clone, participated in experiments and aided in the interpretation and writing of the experiments. PC and PR designed and provided beta cell lines with training and support and revised the manuscript. RS initiated the project (including experiments), revised the manuscript and provided beta cell lines. BOR initiated and supervised the project, designed experiments, wrote the manuscript and is the guarantor of this work. All authors approved the final version of the manuscript.

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References

1. Bennet W, Groth CG, Larsson R, Nilsson B, Korsgren O (2000) Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J Med Sci 105:125–133
2. Arif S, Moore F, Marks K et al (2011) Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated beta-cell death. Diabetes 60:2112–2119
3. Huurman VA, Hilbrands R, Pinkse GG et al (2008) Cellular islet autoimmune associates with clinical outcome of islet cell transplantation. PLoS One 3:e2435
4. Ravassard P, Hazhoy Y, Pechherty S et al (2011) A genetically engineered human pancreatic beta cell line exhibiting glucose-inducible insulin secretion. J Clin Invest 121:3589–3597
5. Carlotti F, Bazuiu M, Kekarainen T et al (2004) Lentiviral vectors efficiently transduce quiescent mature 3T3-L1 adipocytes. Mol Ther 9:209–217
6. Pahl JH, Ruslan SE, Buddingh EP et al (2012) Anti-EGFR antibody cetuximab enhances the cytolytic activity of natural killer cells toward osteosarcoma. Clin Cancer Res 18:432–441
7. Skovera A, Ellis RJ, Varela-Calvino R et al (2008) CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. J Clin Invest 118:3390–3402
8. Borst J, de Vries E, Spits H, de Vries JE, Boylston AW, Matthews EA (1987) Complexity of T cell receptor recognition sites for defined alloantigens. J Immunol 139:1952–1959
9. van der Pol P, Roos A, Berger SP, Daha MR, van Kooten C (2011) Natural IgM antibodies are involved in the activation of complement by hypoxic human tubular cells. Am J Physiol Renal Physiol 300:F932–F940
10. Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, Fibbe WE (2006) Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood 108:2114–2120

11. Mulder A, Kardol M, Blom J, Jolley WB, Melief CJ, Bruning JW (1993) Characterization of two human monoclonal antibodies reactive with HLA-B12 and HLA-B60, respectively, raised by in vitro secondary immunization of peripheral blood lymphocytes. Hum Immunol 36:186–192

12. Roep BO, Arden SD, De Vries RR, Hutton JC (1990) T cell clones from a type-1 diabetes patient respond to insulin secretory granule proteins. Nature 345:632–634

13. Coppieters KT, Dotta F, Amirian N et al (2012) Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 209:51–60