Reaction Patterns of Natural Xenophile Antibodies in Human Sera With Pancreatic Islet Cells and Porcine Lymphocytes

V. Eckstein, W. Müller-Ruchholtz, and K. Ulrichs

THE INCREASING shortage of allogeneic donor organs for clinical transplantation has induced those working in this field to turn their attention to xenotransplantation and suitable donor species. A species which is much favoured at present, and has been used in preliminary clinical trials, is the pig.[1] Among the needed organs, the isolated porcine pancreatic islet appears to have the most realistic chance of being xenografted, since this miniature organ, unlike kidney, heart, or liver, is nonvascularized at the time of transplantation. This means that preformed natural xenophile antibodies (NXA), which bind primarily to vascular endothelial cells and cause hyperacute and acute xenograft rejection of vascularized organs, would be of minor relevance for the survival of the islet xenograft unless the same target antigens are also expressed on other cells. To investigate the reactivity of NXA with islet cells and their crossreactivity with lymphocytes, sera from healthy persons and type I diabetic patients (insulin-dependent diabetes mellitus [IDDM]) were analyzed in indirect immunofluorescence, antibody absorption, and inhibition assays for: (a) their binding pattern with porcine pancreatic islet cells; (b) cytotoxicity; (c) target epitope distribution and specificity; and (d) possible cellular origin. In the case of IDDM patients, the term "NXA" may be misleading, inasmuch, as they very likely represent a mixture of natural and diabetes-dependent crossreacting xenophile antibodies.

MATERIALS AND METHODS

Human Test Sera and Blood Cells

Human sera were collected from 34 healthy persons and 50 IDDM patients. The sera were heat inactivated and diluted 1:2 with phosphate-buffered saline before use. Human peripheral blood lymphocytes (PBL) were obtained by venipuncture, followed by density gradient centrifugation of the heparinized blood. Cells were harvested, washed and prepared according to standard procedures.

Porcine Pancreatic Tissue and Blood Cells

Pancreatic tissue from male and female pigs (9-month-old, 100 to 110 kg dead weight) were obtained from a local abattoir, cut to pieces, snap frozen in liquid nitrogen, and used for the preparation of frozen tissue sections. Porcine peripheral blood cells, ie, lymphocytes/monocytes and erythrocytes, were obtained by collecting heparinized blood during the slaughtering process. Cell separation and preparation were carried out according to a standard protocol for human cells.

Absorption of Human Sera with Porcine Blood Cells

Selected human sera (healthy persons, IDDM patients) were absorbed 3× with either porcine lymphocytes/monocytes (500 μL serum incubated with 10^7 cells, 30 minutes at room temperature, gentle shaking) or with erythrocytes (500 μL serum incubated with 500 μL erythrocytes). Exhaustive absorption was not intended, since determining antibody specificity was the object of the investigation and not complete antibody elimination.

Natural Xenophile Antibody Analysis

Cryostat sections of porcine pancreas were incubated with the human sera (healthy persons, IDDM patients) for 45 minutes, washed, and further incubated with an FITC-conjugated goat antihuman immunoglobulin (Ig), anti-IgG, or anti-IgM antiserum (Dianova, Hamburg, Germany). The reactivity of human NXA was analyzed with a Zeiss ICM-405 fluorescence microscope. The intensity of NXA binding was graded as strongly positive (titer ≥ 1:32), weakly positive (titer ≤ 1:16), or no reactivity.

Pancreatic Target Cell Types

The fluorescence analysis of NXA binding on the porcine pancreas included vascular endothelial cells (VE), ductal epithelial cells (DE), macrophages (MO), endocrine cells (EN), and exocrine cells (EX).

Preincubation of Human Serum NXA with Various Carbohydrates

For the NXA-binding inhibition test, human sera (healthy persons, IDDM patients) were preincubated (30 minutes room temperature, vol/vol) with carbohydrate solutions before being used on porcine pancreas tissue sections. The following mono- and disaccharides were used in a concentration of 1.5, 15, and 150 mmol: *N*-acetyl-mannosamine, melibiose, cellobiose, rhamnose, gentiobiose, lactose, mannose, and saccharose.

Detection of Human Complement Deposition on Porcine Pancreas

A FITC-coupled mouse monoclonal antibody against human complement factor C3 was used to detect whether human complement factors in combination with human immunoglobulins (from IDDM sera) bind to porcine pancreas. In this test, a two-colour immunofluorescence was carried out and human Ig was detected by a goat antihuman-Ig-Cy3 (cyanine fluorochrome) antiserum (Dianova).

From the Institute of Immunology, Medical School of the University, Kiel, Germany.

Address reprint requests to Volker Eckstein, Institute of Immunology, Medical School, Brunswikerstr 4, D-24105 Kiel, Germany.

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Table 1. Inhibition of NXA Binding by Serum Absorption with Porcine Blood Cells

Absorption Status	Porcine Pancreatic Target Cell		
	VE, DE, MO	EX	EN
IDDM serum unabsorbed	1:16	1:2	1:64
Absorbed 1× with PBL	1:8	1:2	1:64
Absorbed 2× with PBL	1:8	1:2	1:64
Absorbed 3× with PBL	1:4	1:2	1:64
Absorbed 1× with ERYTHR	1:8	1:2	1:64
Absorbed 2× with ERYTHR	1:8	1:2	1:64
Absorbed 3× with ERYTHR	1:4	1:2	1:64

PBL: porcine peripheral blood lymphocyte; ERYTHR: porcine erythrocyte; VE: vascular endothelial cell; DE: ductal epithelial cell; MO: macrophage; EX: exocrine tissue; EN: endocrine tissue; IDDM serum: serum from a type I diabetic patient with particularly strong NXA reactivity toward porcine endocrine cells; this serum was representative of four similarly reacting IDDM sera; the absorption effect was tested in indirect immunofluorescence histology (titer).

RESULTS

Cytotoxic Effects of Human NXA on Porcine Pancreatic Tissue

To test whether human NXA that showed binding specificity for porcine pancreatic islets[2] can activate human complement, the deposition of human complement factor C3 was evaluated by two-colour immunofluorescence histology using an anti-C3 monoclonal antibody (MAb). The results of this study, which is not documented in detail by figures, clearly indicate C3 deposition in the case of positive NXA binding. This deposition was seen on all islet target cells, including intraislet VE, and it was completely eliminated by heat inactivating the test serum.

Inhibition of NXA Binding by Serum Absorption with Porcine Blood Cells

The following test was carried out to evaluate a possible target epitope relationship on different porcine cells. An IDDM serum containing NXA against vascular and ductal epithelial cells, macrophages, and islet cells, was repeatedly absorbed with porcine lymphocytes/monocytes or erythrocytes prior to its evaluation in immunofluorescence histology. The results are documented in Table 1 and can be summarized as follows: (1) three serum absorptions with porcine PBL resulted in a stepwise reduction of NXA with specificity for vascular endothelial and ductal epithelial cells and macrophages, but not in a reduction of NXA with specificity for exocrine and endocrine cells; (2) the latter finding was also obtained after absorption with the second cell type, the porcine erythrocyte; and (3) this reaction pattern was seen not only with specially selected IDDM sera but also in islet-reactive sera from normal persons (data not shown).

NXA Binding Inhibition by Serum Preincubation with Carbohydrates

As an extension of earlier studies[3] we wanted to evaluate whether carbohydrate structures are parts of the NXA target epitopes on islet cells. Therefore, human sera (IDDM patients, n = 4) were incubated with eight different mono- and disaccharides of varying concentrations prior to histological evaluation. The results are documented in Table 2 and can be summarized as follows: (1) of the eight carbohydrates tested, only melibiose was capable of reducing NXA binding, as revealed by indirect immunofluorescence histology; (2) reduction of NXA binding was seen on all target cells, including the endocrine islet cells; and (3) this reduction increased with increasing carbohydrate concentration (Table 2 documents the results for 150 mmol only).

Table 2. NXA Binding Inhibition by Serum Preincubation with Carbohydrates

Preincubation with Carbohydrate (150 mmol)	Porcine Pancreatic Target Cell		
	VE, DE, MO	EX	EN
IDDM serum: no preincubation	1:32	1:2	1:32
N-acetyl-mannosamine	1:32	1:2	1:32
Melibiose	**1:4**	**1:2**	**1:4**
Cellobiose	1:32	1:2	1:32
Rhamnose	1:32	1:2	1:32
Gentiobiose	1:32	1:2	1:32
Lactose	1:32	1:2	1:32
Mannose	1:32	1:2	1:32
Saccharose	1:32	1:2	1:32

VE: vascular endothelial cell; DE: ductal epithelial cell; MO: macrophage; EX: exocrine tissue; EN: endocrine tissue; IDDM serum: serum from a type I diabetic patient with particularly strong NXA reactivity toward porcine endocrine cells; this serum was representative of four similarly reacting IDDM sera; preincubation (vol/vol) was performed with three carbohydrate concentrations: 1.5, 15, and 150 mmol; the preincubation effect was tested in indirect immunofluorescence histology (titer). Only the result with 150 mmol is documented above.

DISCUSSION

So far the origin of NXA in IDDM patients is very unclear. As already mentioned, they may represent a mixture of natural and diabetes-dependent crossreacting xenophile antibodies. This can be assumed from our previous observations that NXA from IDDM patients showed stronger binding to porcine endocrine islet cells than NXA from healthy persons.[2] Unless this conflict is solved, the term "NXA" must be handled with caution.

The present study was a continuation of these previous attempts by our laboratory to analyze xenophile antibodies in the serum of IDDM patients.[2] It appeared to be of major interest because eight clinical fetal porcine preislet xenografts have already been performed in IDDM patients during the past 2 years in Stockholm.[1] They may have failed because they were attacked by NXA and graft-induced xenophile antibodies in particular, following maturation of the preislet cells. Our finding that deposition of human complement factor C3 follows NXA binding appears to be an important hint at the cytotoxic potential of these antibodies. If so, antibody elimination prior to porcine islet transplantation should be attempted. Studies along this line, namely to eliminate NXA and to down-

regulate their production by pharmacological manipulation, are presently also being performed in our group.[4–6] Absorption of the IDDM serum with porcine lymphocytes/monocytes and erythrocytes clearly indicated that different target epitopes on different target cell types are recognized by NXA. This is not surprising since NXA represent a pool of antibodies with different specificities.[7,8] Consequently, and this should be pointed out for clinical application, the peripheral porcine blood lymphocyte may substitute as a target cell for vascular endothelial cells, but not for endocrine islet cells in pretransplant NXA screening assays.

As an extension of previous studies by our group, it was found that NXA showed binding specificity for carbohydrate structures, particularly for the disaccharide melibiose.[3] The experiments reported here were performed according to the original protocol using IDDM sera with strong anti-islet NXA reactivity. Inhibition of NXA binding by preincubation was incomplete, possibly due to residual NXA with specificity for: (a) other carbohydrates, which were not included in our study; or (b) glycoproteins, which are detectable on porcine endothelial cells.[9] Our finding that melibiose is a powerful NXA inhibitor has been confirmed in the meantime by other groups which used either melibiose or related carbohydrates with galactose residues.[10,11] Generally, healthy persons showed an identical antibody reduction pattern, though with quantitative differences, depending on the amount of reactive antibodies.

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REFERENCES

1. Groth CG, Andersson A, Korsgren O, et al: Transplant Proc 25:970, 1993

2. Eckstein V, Ulrichs K, Meincke G, et al: Transplant Proc 24:681, 1992

3. Laus R, Ulrichs K, Müller-Ruchholtz W: Int Arch Allergy Appl Immunol 85:201, 1988

4. Ulrichs K, Kaitschick J, Bartlett R, et al: Transplant Proc 24:718, 1992

5. Breitkreuz A, Ulrichs K, Eckstein V, et al: Transplant Proc 25:416, 1993

6. Ulrichs K, Wang H, Müller-Ruchholtz W: Transplant Proc (this issue)

7. Milgrom F: In Hardy MA (ed): Xenograft '25. Amsterdam, Excerpta Medica, 1989, p 149

8. Turman MA, Casali P, Notkins AL, et al: Transplantation 52:710, 1991

9. Platt JL, Vercellotti GM, Dalmasso AP, et al: Immunol Today 11:450, 1990

10. Good AH, Cooper DKC, Malcolm AJ, et al: Transplant Proc 24:559, 1992

11. Thibaudeau K, Anegon I, Lemauff B, et al: Transplant Proc 26:(this issue), 1994