# Natural Xenophile Antibodies From Sera of Type I Diabetic Patients Differ Strongly in Their Reactivity Against Various Porcine Pancreatic Cells

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TRANSPLANTATION of xenogeneic porcine pancreatic islets is considered an alternative therapy for patients suffering from insulin-dependent diabetes mellitus (IDDM), to make up for shortage of donor organ. Successful xenografting, however, requires elimination of preexisting natural xenophile antibodies (NXA), which are responsible for acute and hyperacute graft rejection.[1] In this study we analyzed NXA in sera from 50 IDDM patients and 34 human control sera for their reactivity with pancreatic tissue from seven different pig races.

## MATERIALS AND METHODS

### Sera

Test sera were collected from 50 type I diabetic patients of both sexes, ranging in age from 16 to 67 years, with diabetes known for more than 2 years. Thirty-four control sera were obtained from healthy human individuals of both sexes ranging in age from 25 to 62 years. The sera were heat inactivated and diluted 1:2 with phosphate-buffered saline before use.

### Porcine Pancreatic Tissue

The pancreata (splenic lobe) of 21 male or female pigs (9 months old, 100-110 kg body weight) of seven different races were collected at local slaughterhouses, cut to pieces, snap frozen in liquid nitrogen and used for the preparation of frozen tissue sections.

### Pig Races

The following pig races were used: Pietrain, Duroc, Hampshire, Deutsche Landrasse, Belgische Landrasse, Hampshire × Belgische Landrasse, and Göttinger Minipig.

### Natural Xenophile Antibody Analysis

Cryostat sections of the pancreas were incubated with the sera (from diabetic patients or human controls) for 45 minutes, washed, and further incubated (45 minutes) with FITC-conjugated anti-immunoglobulin (Ig), anti-IgG, or anti-IgM secondary antibodies. Reactivity for NXA was analyzed with a Zeiss ICM-405 fluorescence microscope.

### Fluorescence Grading

Intensity of NXA reactivity with the various pancreatic cells and NXA serum titers was correlated as follows: strongly positive reactivity was ++++ (1:128), +++ (1:64), and ++ (1:32); weakly positive reactivity was + (up to 1:16); negative reactivity, ie, no titer, was —.

### Pancreatic Cell Types

Fluorescence analysis included vascular endothelial cells (VE), ductal epithelial cells (DE), macrophages (MO), endocrine cells (EN), and exocrine cells (EX).

## RESULTS

### Distribution Pattern of Natural Xenophile Antibodies in Insulin-Dependent Diabetes Mellitus Patients and Human Control Sera

According to fluorescence microscopy, all 50 IDDM sera and all 34 human control sera contain NXA reactive with one or the other of the various pancreatic cell types, ie, VE, DE, MO, EN, and/or EX. The intensity of binding varies from cell type to cell type.

Comparing the two serum donor groups, the following observations can be made (Table 1): (1) In general, reactivity with VE, DE, and MO tends to be stronger than reactivity with EN. Reactivity with VE, DE, and MO does not correlate with strong reactivity with EN. (2) Both serum types, IDDM and controls, have a similar distribution pattern with regard to their reactivity with VE, DE, and MO. (3) Sera from IDDM and controls react differently with EN; in the IDDM group, the number of strongly reacting sera is significantly increased (30% versus 4%). In addition, this group contains a significantly smaller number of NXA-negative sera than the control group (19% versus 50%).

### Elimination of Vascular Endothelial Cells as an Intraislet Natural Xenophile Antibody Target

Vascular endothelial cells can be eliminated or structurally disintegrated by culturing isolated Duroc islets over a period of 24 to 48 hours at 37°C. Disintegration of VE was demonstrated by staining fresh and cultured islets with the anti-porcine major histocompatibility complex class II monoclonal antibody MSA3. Whereas in fresh isolated islets intact VE form a distinct three-dimensional network, only a few remaining fluorescent cell fragments or no fluorescent cells at all can be detected in cultured islets.

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**Table 1. Percentage of Sera Containing Natural Xenophile Antibodies Against the Various Cell Types in the Porcine Pancreas**

| | Reactivity Grading | | |
|---|---|---|---|
| | Strongly Positive | Weakly Positive | Negative |
| Human controls (n = 34) | | | |
| VE, DE, MO | 30 | 65 | 5 |
| EN | 4 | 46 | 50 |
| IDDM patients (n = 50) | | | |
| VE, DE, MO | 40 | 56 | 4 |
| EN | 30 | 51 | 19 |

Note: Sera were harvested from healthy human controls and IDDM patients and tested on Duroc-17 pancreas cryostat sections in indirect immunofluorescence (n=5).

Abbreviations: VE, vascular endothelial cells; DE, ductal epithelial cells; MO, macrophages; EN, endocrine islet cells.

### Natural Xenophile Antibody Reactivity With Endocrine Islet Cells

Table 2 documents for the human control group that NXA reactivity with EN, divided into "strongly positive", "weakly positive", and "negative" reactivity, differs markedly between the seven pig races and considerably among individuals within the same race. The right part of Table 2 clearly indicates that there are NXA-negative sera. Similar studies with IDDM sera are in progress.

### Isotypes of Natural Xenophile Antibodies

Adult human NXA are predominantly of IgG and rarely of IgM isotypes (IgG >> IgM). This holds equally for both groups: human control sera and sera of diabetics. Analysis of NXA in the cord blood of newborn children reveals exclusively IgG (of presumably maternal origin) and no IgM isotypes, whereas sera of children older than 1 year show a reactivity pattern and distribution of NXA isotypes similar to adult human beings (IgG >> IgM).

## DISCUSSION

In vivo and in vitro studies have revealed that normal human sera contain NXA against antigens present on various porcine cells, eg, EN, lymphocytes, erythrocytes, and others.[1,2] In this study, we extended these investigations to cells of pancreatic tissue, including the EN islet cells. In contrast to the EX pancreatic cells, eg, VE, DE, and MO, these are of particular interest in connection with xenogeneic islet transplantation for IDDM patients.[3] Therefore, a thorough NXA analysis is presented here for the first time.

Whether NXA are a product of antigenic sensitization or are germ-line determined, or whether they are a mixture of both, still remains a matter of discussion.[4,5] The possibility that NXA are solely a product of species-specific sensitization, eg, eating of pork, is unlikely, because sera from Israeli volunteers who never consumed pork showed a similar NXA distribution pattern to that of the human control sera (unpublished data). To date, our analysis of

**Table 2. Reactivity of Natural Xenophile Antibodies in Human Control Sera With Pancreatic Islet Cells From Various Pig Races and Individuals**

| Pig Race and Individual Number | Strongly Positive Sera (%) | Weakly Positive Sera (%) | Negative Sera (%) | Sera (n) |
|---|---|---|---|---|
| PI-1 | 6 | 67 | 27 | 33 |
| PI-2 | 33 | 52 | 15 | 33 |
| PI-12 | 0 | 70 | 30 | 33 |
| PI-21 | 36 | 52 | 12 | 33 |
| DU-9 | 13 | 41 | 47 | 32 |
| DU-14 | 21 | 70 | 9 | 33 |
| DU-17/1 | 4 | 46 | 50 | 32 |
| DU-17/2 | 4 | 45 | 51 | 33 |
| HA-4 | 7 | 39 | 55 | 31 |
| HA-5 | 7 | 77 | 16 | 31 |
| HA-6 | 12 | 61 | 27 | 33 |
| DL-17 | 9 | 67 | 24 | 33 |
| DL-18 | 15 | 46 | 40 | 33 |
| DL-27 | 6 | 40 | 55 | 33 |
| BL-4 | 21 | 61 | 18 | 33 |
| BL-7 | 12 | 67 | 21 | 33 |
| BL-8 | 22 | 47 | 31 | 32 |
| HABL-18 | 33 | 52 | 15 | 33 |
| HABL-19 | 24 | 55 | 21 | 33 |
| HABL-20 | 30 | 64 | 6 | 33 |
| MP-1 | 3 | 34 | 63 | 32 |
| MP-2 | 0 | 61 | 39 | 33 |
| MP-3 | 0 | 42 | 58 | 33 |

Note: Sera were tested on frozen tissue sections in indirect immunofluorescence.

sera from newborn and young children can only address when both NXA isotypes, IgG and in particular IgM, appear, but cannot answer the question of the inducing event(s).

The comparatively strong reactivity of NXA of diabetics with EN may suggest that circulating autoantibodies cross-react with xenogeneic antigen. However, all IDDM patients were autoantibody-negative at the time of testing, which precludes this possibility. It may be considered that the pathogenic autoimmune process, leading to islet destruction, does not only activate autoreactive B-cell clones, but also xenoreactive ones.

The observation that 19% of the sera from diabetics are negative for NXA against porcine EN islet cells clearly speaks in favor of isolated xenogeneic islet transplantation. However, most of the sera show strong reactivity with VE not only in the exocrine but also in the endocrine tissue. Therefore, the process of eliminating intra-islet VE by, eg, a pretransplant culturing period, appears to be very important. It will not only abolish an NXA target but also a potentially immunogenic structure, since porcine VE has been shown to strongly express major histocompatibility complex class II molecules.[6]

The 30% strongly positive and 51% weakly positive IDDM sera (against EN) could be a matter of great concern. How cytotoxic they are remains to be elucidated. Efforts to characterize the NXA target antigen(s) and thus

prepare the basis for developing immunomanipulatory strategies are required. So far, the availability of a nearly unrestricted number of porcine islet donor races opens the possibility of finding optimum donor/recipient combinations.

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## REFERENCES

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

2. Hammer C: Transplant Proc 19:4443, 1987

3. Andersson A, Groth CG, Korsgren O, et al: Transplant Proc 24:677, 1992

4. Rajewsky K: Prog Immunol 7:397, 1989

5. Milgrom F: In: Hardy MA (ed): Xenograft 25. Amsterdam, The Netherlands, Elsevier Science, 1989, p 149

6. Bretzel RG, Flesch BK, Hering BJ, et al: In: Federlin K, Bretzel RG, Hering BJ, et al (eds): Methods in Islet Transplantation Research. New York, NY, Thieme, 1990, p 128