Long-Term Specific Suppression of Antibody Production by Plasmapheresis and a Short-Term Course of Cyclophosphamide

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PLASMAPHERESIS in conjunction with a defined, time-related course of subsequent pulse cyclophosphamide (CY) injections has been shown to be very effective in treating patients who suffer from severe forms of systemic lupus erythematosus (SLE).[1-3] It was the aim of this study to analyze the effect of this clinical protocol on the formation of natural xenophile antibodies (NXA) against porcine pancreatic islet cells, which are known to be responsible for acute and hyperacute rejection of xenografts.

Table 1. Serum Titers Against Various Cellular Antigeneic Structures of Porcine Pancreas

	Serum Antibody Reactivity				
	Cells (NXA)			Nuclei (ANA)	
Titer	VE, DE, MO	EX	EN	EX	EN
(a) Healthy human control					
1:2	+++	—	+/++	—	—
1:4	++	—	+	—	—
1:8	+	—	—	—	—
1:16	—	—	—	—	—
(b) SLE patient S.R.*					
1:2	—	—	—	++++	++++
1:4	—	—	—	++/+++	++++
1:8	—	—	—	++	+++
1:16	—	—	—	+/++	++
1:32	—	—	—	+	++
1:64	—	—	—	—	+
1:128	—	—	—	—	—
(c) SLE patient S.R.†					
1:2	—	—	—	+	+/++
1:4	—	—	—	—	—
1:8	—	—	—	—	—
1:16	—	—	—	—	—
1:32	—	—	—	—	—
1:64	—	—	—	—	—
1:128	—	—	—	—	—
(d) SLE patient S.R.‡					
1:2	+/++	—	—	+	+
1:4	+/++	—	—	+	+
1:8	+	—	—	—	—
1:16	—	—	—	—	—
1:32	—	—	—	—	—
1:64	—	—	—	—	—
1:128	—	—	—	—	—

* Thirty-year-old woman before combined treatment (serum 9316).
† Thirty-year-old woman immediately (2 days) after combined treatment (serum 9333).
‡ Thirty-year-old woman long term (7 months) after combined treatment (serum 9963).

MATERIALS AND METHODS

Serum Donors

Sera of five SLE patients, being positive for antinuclear antibodies (ANA) were sampled routinely before, immediately after, and long-term after combined treatment, consisting of plasmapheresis and CY.[1] Sera of 34 healthy humans served as controls. Immediately before testing in indirect immunofluorescence, sera were heat inactivated and titrated twofold with phosphate-buffered saline (PBS).

Porcine Pancreatic Tissue

Porcine pancreata were obtained from local slaughterhouses, cut to pieces, snap frozen in liquid nitrogen, and prepared as frozen tissue section for immune histology.

Standard Immunofluorescence

This was carried out using the sera of SLE patients or healthy humans as a primary antibody source. Fluorescein isothiocyanate (FITC)-conjugated goat-antihuman Ig, IgG, and IgM served as secondary antibodies. Fluorescence was evaluated with a Zeiss ICM 405 microscope.

Fluorescence Grading

Note the following: ++++, +++, ++, = strongly positive; + = weakly positive; − = negative.

Pancreatic Cell Types

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

RESULTS

The results can be summarized as follows: (1) Sera of healthy humans contain NXA that react with the various porcine pancreatic cells, for example, VE, DE, MO, and EN (Table 1a). These sera are usually negative for ANA. (2) In contrast to healthy humans and before combined treatment, sera of SLE patients react strongly positive for ANA, but they are negative for NXA on the various

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porcine pancreatic cells (Table 1b). (3) Immediately, that is, 2 days after patient treatment, the content of ANA is significantly reduced in these sera while NXA are still undetectable (Table 1c). (4) Long-term (7 months) after patient treatment, only a marginal reoccurrence of ANA is observed. However, NXA reoccur and reach normal titer ranges (Table 1d). (5) NXA are of IgG rather than IgM isotype, as the analysis with FITC-conjugated anti-IgG and anti-IgM secondary antibodies revealed (not documented).

CONCLUSIONS

From our results we want to draw the following conclusions: (1) Plasmapheresis combined with a short-term course of the alkylating agent CY appears to be very effective in eliminating activated B-cell clones, but rather ineffective in eliminating "resting" clones, such as NXA clones. (2) In the context of xenotransplantation, this clinical protocol could be particularly effective in eliminating those antibodies, which may be produced by xenograft-activated B cells.

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