

### Definite proof for the crucial role of xenobodies in hyperacute rejection in the rat/guinea-pig model

H. Grimm, P. Mages, G. Lindemann and L. Ermer  
Department of General and Thoracic Surgery, University of  
Giessen, Klinikstrasse 29, D-35392 Giessen, Germany

**Introduction:** There is still controversy if xenoreactive antibodies play a role in hyperacute rejection in the rat/guinea-pig xenotransplant model. Alternatively, rejection may be mediated by the antibody-independent alternative pathway of complement activation. This study was done to clarify this issue.

**Methods:** Inbred guinea-pigs (BNF Giessen, 250–300 g) and inbred rats (Sprague-Dawley, Hannover, 220–250 g) served as donors and recipients in a cardiac xenotransplant model. Prior to transplantation plasmapheresis with one of two microfilters (Fresenius SPS 1003 or SPS 9002) was performed. Xenobodies (modified ELISA according to Leventhal), total IgM and IgG (radial immunodiffusion according to Mancini) and complement activity (C'H 50) (complement binding reaction according to Meyer) were assayed prior to and after plasmapheresis and after rejection. The rejected grafts were examined immunohistochemically employing FITC-marked monoclonal antibodies against IgM, IgG and C 3.

**Results:** Complement activity was equally reduced by each of the microfilters to less than 10 C'H 50. Xenobodies proved to be mainly of IgM isotype. Plasmapheresis with SPS 1003 reduced IgM to 26 per cent and with SPS 9002 to 50 per cent their previous levels ( $P < 0.001$ ). Rejection time was  $576 \pm 32$  min (SPS 1003) and  $300 \pm 21$  min (SPS 9002) ( $P < 0.001$ ). IgM and to a lesser degree C 3 were shown to be bound to the graft.

**Conclusions:** With the complement activity equally reduced by either filter, rejection time was inversely related to the residual IgM level. This demonstrates without doubt, that xenobodies do play an important role also in rat/guinea-pig xenotransplantation. These results do not exclude the involvement of the alternative pathway, but underline the importance of the antibody dependent activation of the classical pathway of complement activation in this model.

### Lipid mediated modification of allo-rejection

H. Grimm, J. Schott, C. Blecher, R. Bohle, C. Papavassilis and H. Krämer  
Department of General and Thoracic Surgery,  
University of Giessen, Klinikstrasse 29, D-35392 Giessen,  
Germany

**Introduction:** *n-3* and *n-6* fatty acids, precursors of immunomodulating lipid mediators (leukotrienes, thromboxanes), are claimed to influence the immune system. The immunological impact of the ratio (*n-3/n-6*) of these polyunsaturated fatty acids has not yet been clarified.

**Methods:** Inbred PVG (RT 1<sup>h</sup>) and Wistar/Kyoto rats (RT 1<sup>k</sup>) served as donors and recipients in a heterotopic cardiac allotransplant model. Via a central venous catheter, fish oil (*n-3/n-6-7-6/1*), safflower oil (1/370) or a fish/safflower oil-1:1-mixture (1/2-1) were continuously infused (9 g fat/kg b wt/day) until rejection was complete. Controls received saline. Rejected grafts were examined immunohistologically (MRC OX-19, W 3/25, MRC OX-8, MRC OX-1, MRC OX-33, W 3/13). Ionophore-stimulated leukotriene and thromboxane secretory profile from isolated PMN and thrombocytes were measured by HPLC. Mitogen stimulated  $\alpha$ -TNF and IL-6 secretory capacity from isolated peripheral blood mononuclear cells were measured by ELISA.

**Results:** Rejection time was 7-8 days in the controls and 6-7 days in the fish/safflower oil mixture group. Rejection time was significantly increased in the fish oil (12-3 days) and the safflower oil groups (13-3 days) ( $P < 0.01$ ). In the latter groups the number of cells infiltrating the graft was reduced to approximately 50 per cent,  $\alpha$ -TNF and IL-6 release to around 45 per cent. Leukotriene B5 and thromboxane A3 (*n-3* derivatives) were exclusively produced in the fish oil group.

**Conclusions:** Lipids are not generally immunosuppressive. The ratio of *n-3* and *n-6* fatty acids is decisive. A balanced ratio (fish oil/safflower oil mixture) is immunoneutral. A preponderance of either *n-3* or *n-6* fatty acids has an immunosuppressive effect by reduction of the cyto-

kine release, of the infiltration and proliferation of immunocompetent cells and the modification of lipid mediators.

### Isolation of islets of Langerhans: improvement of the isolation technique using the pig model

A. Heiser, W. Müller-Ruchholtz and K. Ulrichs  
Institute of Immunology, University of Kiel, Michaelisstrasse 5, S-24105  
Kiel; Department of General Surgery, University of Würzburg,  
Germany

During the last few years, interest in pancreatic islet transplantation for the cure of type 1 diabetes has increased markedly. A serious barrier to clinical islet transplantation is the isolation of a sufficient mass of viable and functional islets. We used a porcine islet isolation model to examine various parameters of the isolation procedure and to improve isolation technique.

**Results:** (a) Reduction of mechanical stress during the isolation procedure allowed an increased islet yield (880 vs 95 islets/g organ). (b) A higher islet yield ( $2352 \pm 2619$  islet /g organ) was obtained from adult pigs (>24 months) than from juvenile animals (<14 months;  $996 \pm 1542$  islets/g organ). (c) Some pig races were more suitable than others for islet isolation, e.g. German Landrace gave an islet yield of  $687 \pm 515$  islets/g organ compared to  $2173 \pm 301$  islets/g organ obtained from Pietrain. (d) An individual strong increase in trypsin activity (>1.5 U/ml) correlated with poor islet yield ( $507 \pm 739$  islets/g organ), whereas low trypsin activity (<1.5 U/ml) always correlated with high islet yield ( $6795 \pm 3697$  islets/g of organ). (e) Addition of the protease inhibitor Pefabloc to the isolation medium results in low trypsin activity ( $0.35 \pm 0.15$  U/ml) and reproducible high islet yields ( $8395 \pm 3553$  islets/g of organ).

**Conclusion:** In view of the particular fragility of porcine islets, improvement of the isolation technique, the choice of donor and better control of the enzymatic process are important factors for successful porcine islet isolation. The similarity of the technical problems encountered in isolated porcine and human islets and the physiological similarity of the porcine and human pancreas will probably allow us to transfer this knowledge from porcine to human islet isolation.

### The relationship between immunosuppression and high-dose steroid in the treatment of acute rejection

C. Jiménez, J. Bercedo, E. Moreno, C. Moreno, J.C. Palomo, C. Loinaz and J. Seoane  
General and Digestive Surgery  
Department, Liver Transplantation Unit, '12 de Octubre'  
Hospital, Ctra. Andalucía Km 5.4, Madrid, Spain

**Aim:** High-dose steroid resistance and acute rejection have been related with several aspects of the immunosuppressive treatment prior to rejection. The aim of this study is to analyse this relationship and the implication in orthotopic liver transplant (OLT) patients.

**Materials and methods:** Between April 1986 and March 1992 we performed 258 OLT on 205 patients: 176 on adults and 29 on infants. We have obtained 279 biopsies of 108 OLT with the diagnosis of acute rejection. Of these, we have studied 106 acute rejection episodes in 66 patients (72 OLT) with the following features: (a) biopsy of acute rejection; (b) short courses of high-dose steroids (10–15 mg/kg/24 h); (c) steroid resistance. The data obtained from two groups (A: resistant, B: non-resistant) were compared.

**Results:** The postoperative immunosuppressive treatment was: steroids + cyclosporin + azathioprine (A: 86.11 per cent; B: 72.2 per cent), steroids + cyclosporin + antilymphocyte globulin (A: 11.11 per cent; B: 16.67 per cent), steroids + cyclosporin (A: 2.78 per cent; B: 11.11 per cent). The immunosuppression prior to the rejection episode was: steroids + cyclosporin + azathioprine (A: 67.8 per cent; B: 65.9 per cent), steroids + cyclosporin + antilymphocyte globulin (A: 3.39 per cent; B: 6.38 per cent), steroids + cyclosporin (A: 28.8 per cent; B: 27.6 per cent). The 24-h dosage when the rejection was diagnosed was: steroid (A: 33.94 mg; B: 33.72 mg), azathioprine (A: 62.63 mg;