

PROLONGED COLD ISCHEMIA LEADS TO ENHANCED IMMUNOGENICITY OF THE GRAFT DUE TO THE REORGANISATION OF THE PROTEASOME COMPLEX

Intention

Ischemia/reperfusion injury (IRI) has a major impact on short-term and long-term renal allograft survival. For a long time, IRI was seen only as a risk factor for delayed graft function (DGF) which is a common clinical situation following kidney transplantation associated with enhanced risk of graft loss and acute rejection. Recent studies revealed, however, that IRI is also an independent risk factor for development of chronic allograft nephropathy (CAN). Actually, it is speculated that synergistically with other donor factors, like age, hypertension and brain death, IRI determines the pre-operative damage of the graft but also the allograft immunogenicity that consequently influences long-term outcome.

In a F344 to LEWIS rat kidney transplantation model, kidneys were subjected to 20 min. or 24 hours of cold ischemia. Recipients were sacrificed 12 hrs after engraftment. A customized cDNA microarray with 820 inflammation and apoptosis related target genes was used for gene expression analysis. Native F344 kidneys served as controls. Gene expression was verified by real-time RT-PCR and protein expression by Western Blot. The full data set consisting of 780 genes was used for data processing. Hierarchical clustering was performed applying average linking, clustering using the Cluster software described in Eisen et al. (http://www.scripps.edu/~eisen/). Statistical significance of gene expression was analyzed by using t-tests of unpaired data and the computer software SAM (Significance Analysis of Microarrays; SAM, http://www-stat.stanford.edu/~tibs/sam/).

Material and Methods

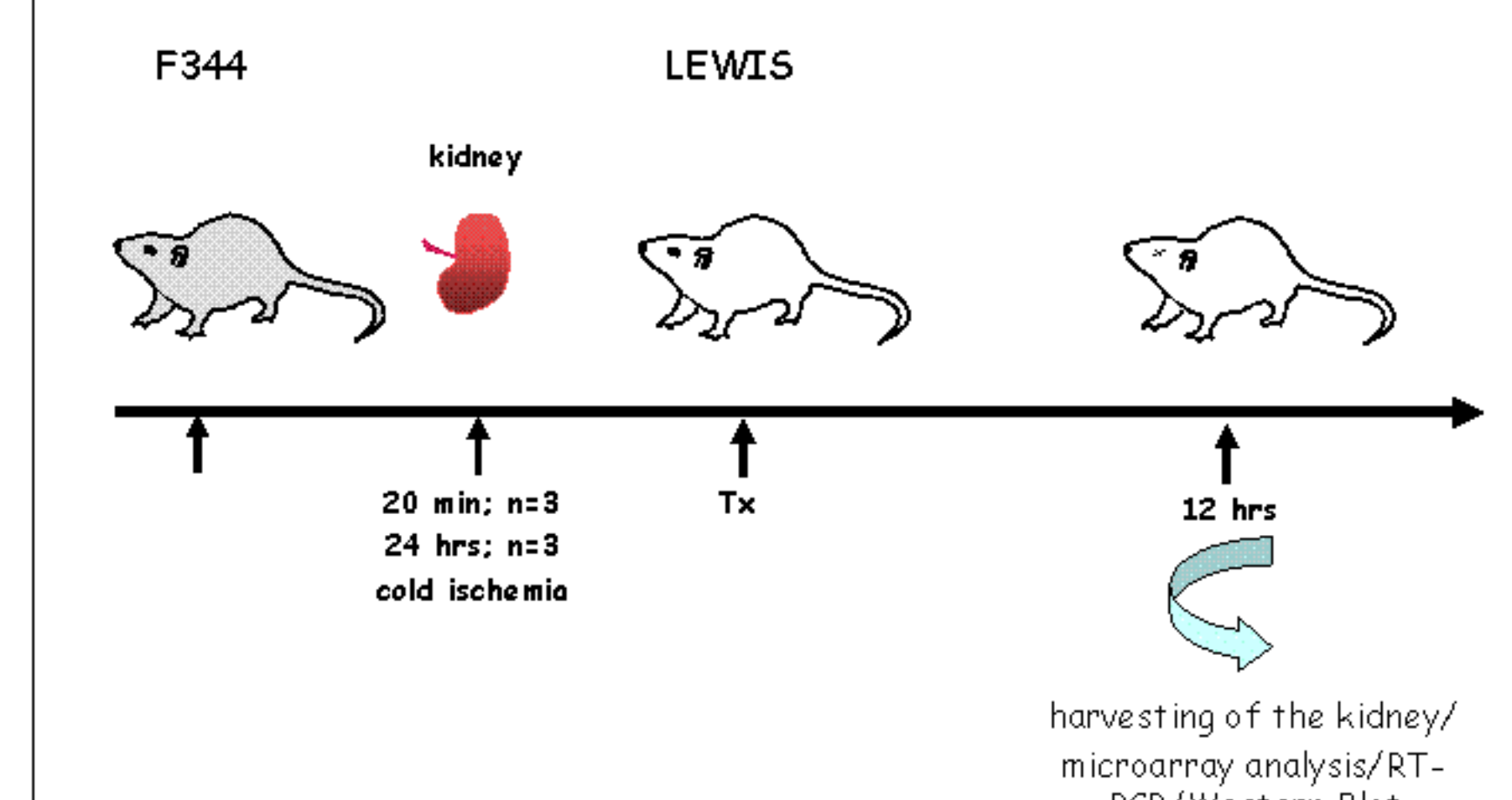


Fig. 1: Rat model. 3 animals/cold ischemia were used. Tx, kidney transplantation.

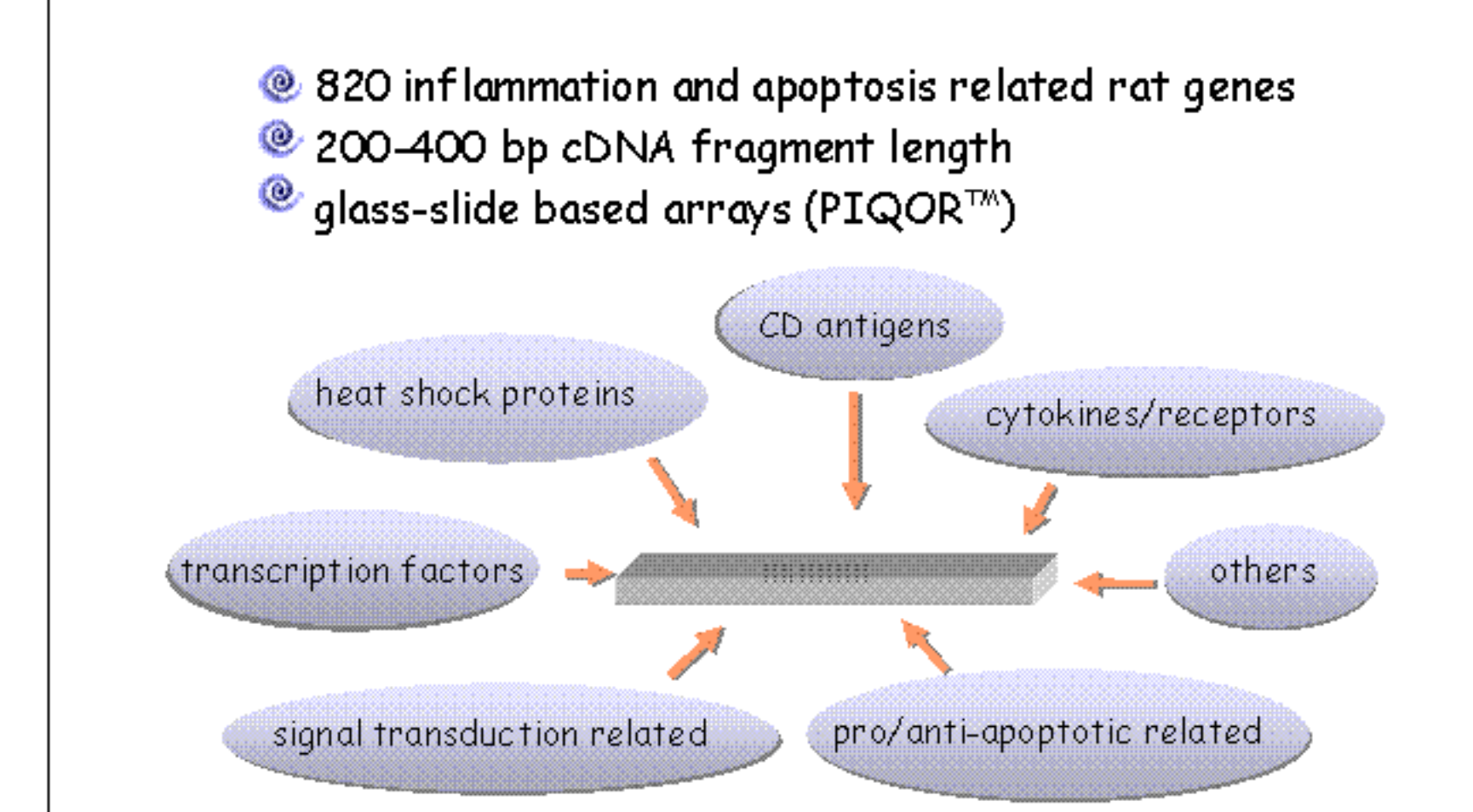


Fig. 2: cDNA microarray design.

Results

Hierarchical clustering of candidate genes

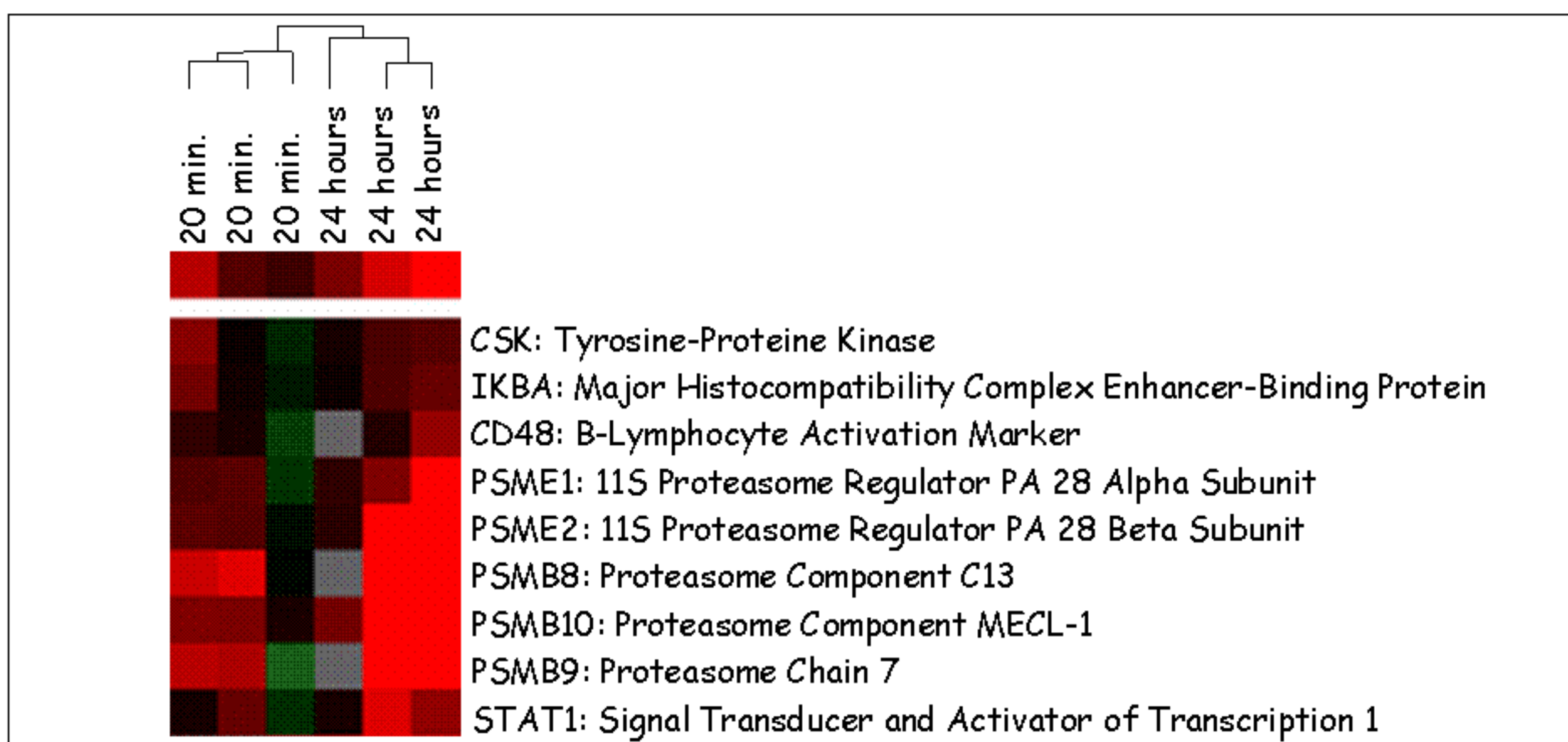


Fig. 3: Gene expression dendrogram showing hierarchical clustering of 9 induced genes during IRI in rat kidneys undergoing either 20 min. or 24 hours of cold ischemia after an observation period of 12 hours. 80% of genes detectable in all experiments were considered for analysis representing in total 139 genes.

Number of regulated genes

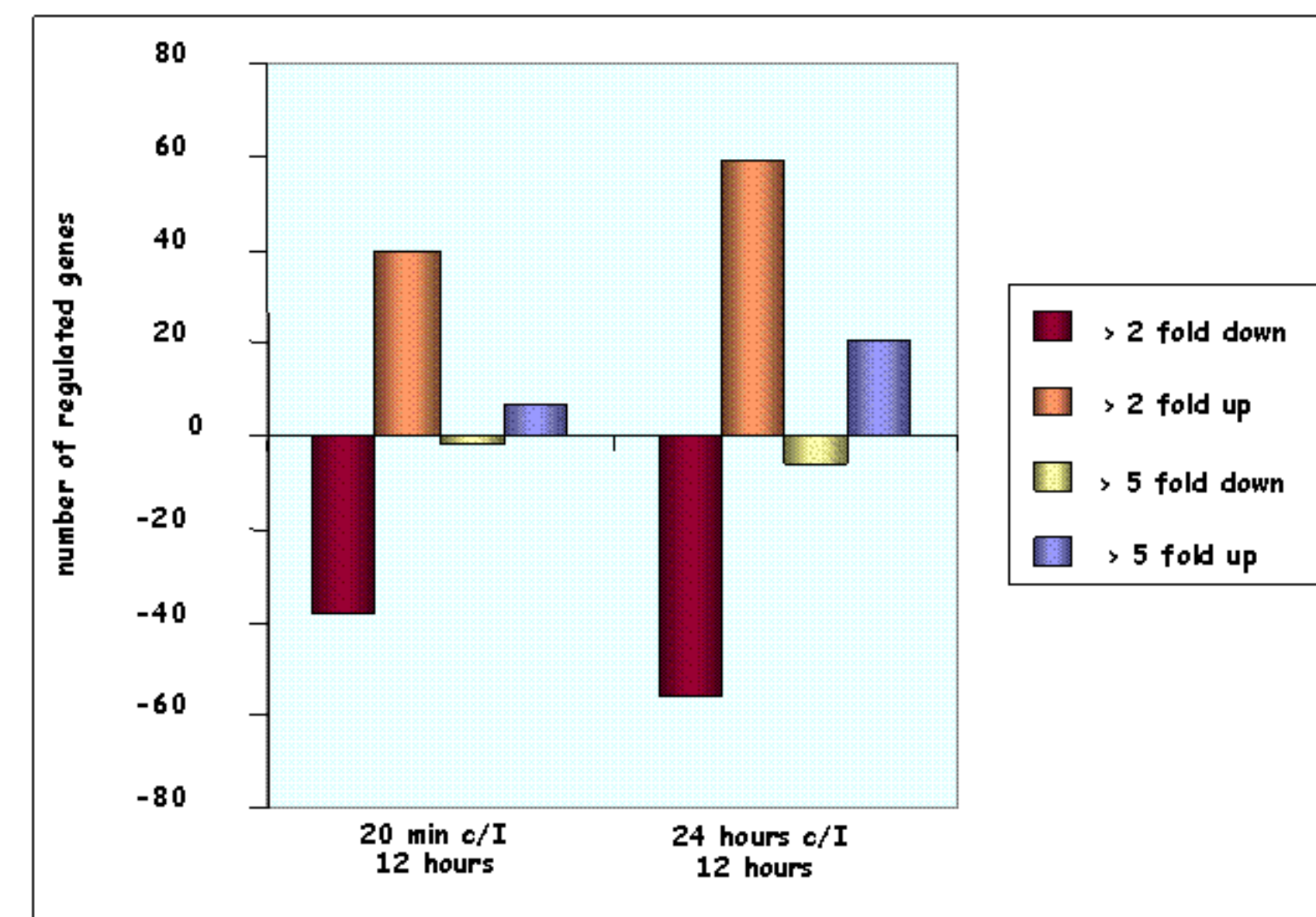


Fig. 4: Number of >2 fold up/down and >5 fold up/down differential expressed genes in all experimental groups. Prolonged cold ischemia of 24 hours leads to a higher number of regulated genes which could be observed short term (12 hours) post Tx. c/I = cold ischemia

Verification of gene expression by real-time RT-PCR

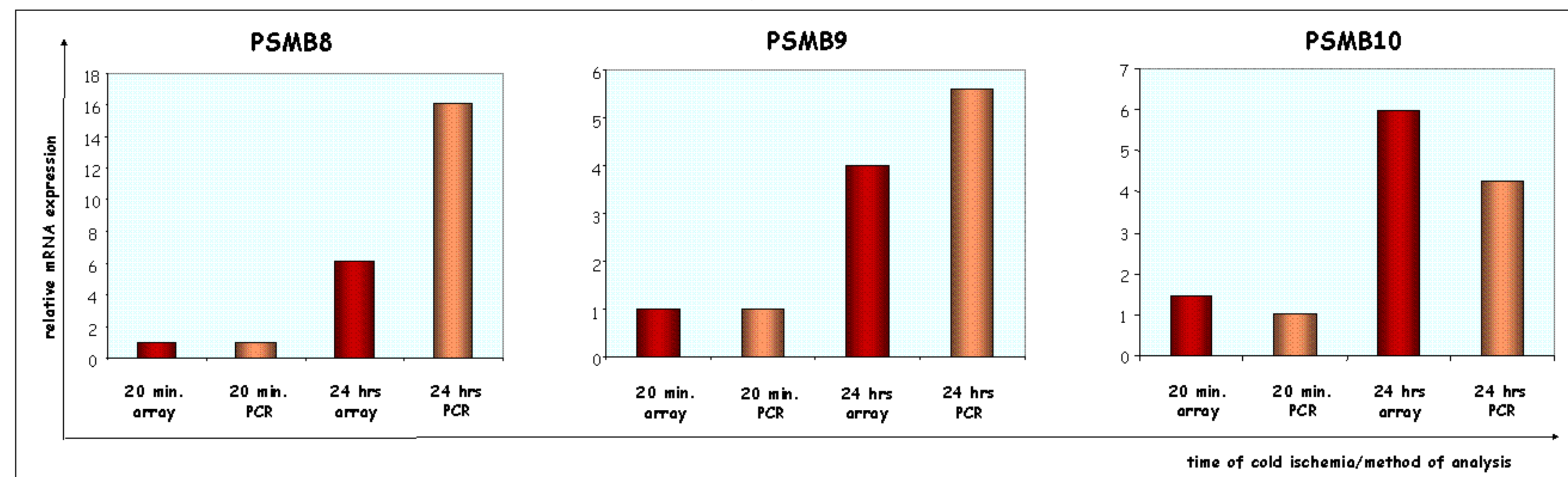


Fig. 5: Comparative gene expression analysis between cDNA microarray and real-time RT-PCR data. The relative mRNA expression is shown for PSMB8 (LMP7), PSMB9 (LMP2) and PSMB10 (MECL-1) compared to the expression profile gained by cDNA microarray analyzed after 12 hrs of engraftment. There was a good correlation between PCR findings and microarray expression data in terms of direction of transcriptional activity.

Measurement of protein expression by Western Blot analysis

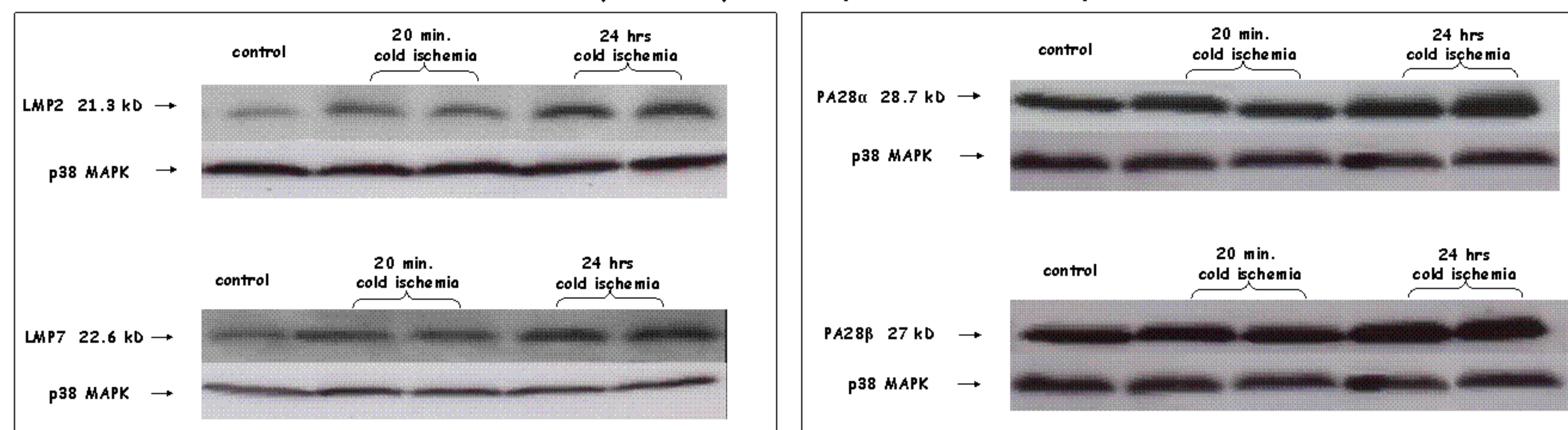


Fig. 6: Western Blot analysis of the immunoproteasomes LMP2 (PSMB9), LMP7 (PSMB8) the proteasome activator subunits PA28 α/β (PSME1, PSME2) in rat kidney allografts undergoing 20 min. or 24 hours of cold ischemia. A native F344 kidney served as control. The staining of p38 MAPK served as internal control.

Discussion

Proteasomes are the main multicatalytic proteinase complex involved in stress response (e.g. NF κ B activation), apoptosis and in the generation of intracellular antigens. The antigenic peptides presented on major histocompatibility complex (MHC) class I molecules to cytotoxic T cells are generated in the cytosol by the 20S proteasome which represents the core structure of the proteasome, made up of seven different α and β subunits each. In mammalian cells IFN γ leads to the induction of new proteasome β subunits LMP2 (PSMB9), LMP7 (PSMB8) and MECL-1 (PSMB10) which replace the respective constitutive catalytic subunits χ (PSMB5), γ (PSMB6), and ζ (PSMB7) during de novo assembly of proteasomes (1,2). Furthermore mammalian cells contain a proteasome activator called PA28 (also known as 11S regulator) composed of α and β subunits which is likewise induced by IFN γ but not part of the 20S proteasome itself (Fig.7). Upon stimulation of cells with IFN γ the expression of immunoproteasomes and PA28 is induced resulting in the enhancement of peptide degradation by the 20S proteasome or enlargement of the peptide repertoire presented to CD8 $^{+}$ T cells.

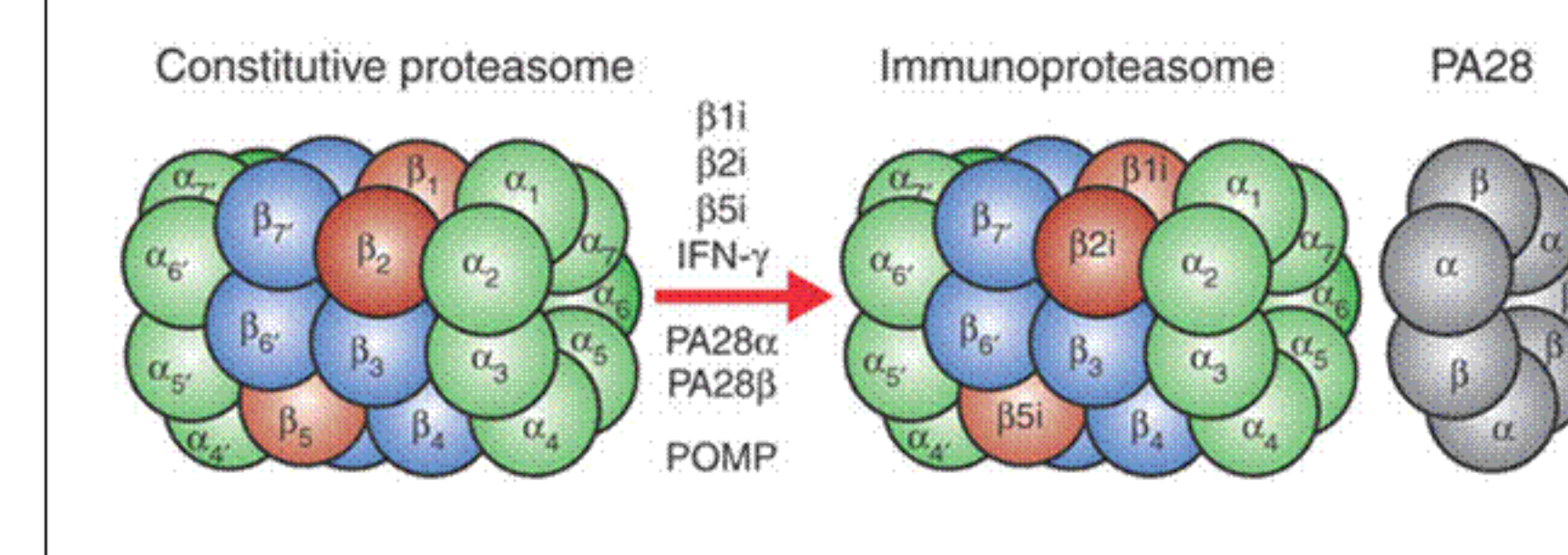


Fig. 7: Formation of immunoproteasomes (adapted from Kloetzel P, Nature Immunology 2004)

We have uncovered the induction of all three IFN γ inducible proteasome subunits (LMP2, LMP7, MECL-1) and of both PA28 α/β subunits (PSME1 and PSME2) in grafts undergoing prolonged cold ischemia (24 hrs) by cDNA microarray analysis and confirmed the heightened expression at the protein level (Fig. 5,6). These so called immunoproteasomes LMP2, LMP7 and MECL-1 appear to alter the cleavage profile of proteasomes resulting in the usage of specific cleavage sites leading to a transition of generated peptides within the peptide pool (3,4).

Conclusion

This in turn may lead to a re-organization of the proteasomes resulting in a change of antigen presentation as PA28 α/β has been demonstrated to alter polypeptide fragmentation in vitro (5). Based on our observation, we hypothesize that induction of LMP2, LMP7 and MECL-1 subunits of immunoproteasomes following prolonged ischemic time alters the allograft "immunogenicity" by inducing NF κ B activation and more efficient generation of allogeneic T-cell epitopes presented to CD8 $^{+}$ T cells resulting in an enhanced antigen-dependent alloimmune response and acceleration of chronic allograft injury. Therefore the targeting of proteasomes and NF κ B activity by specific clinically approved proteasome inhibitors offers a new and reliable approach for the treatment of post-ischemic injury in the near future.

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 3. Schwarz K, Eggers M, Soza A, Koszinowski UH, Kloetzel PM, Groethtrup M. The proteasome regulator PA28alpha/beta can enhance antigen presentation without affecting 20S proteasome subunit composition. Eur J Immunol. 2000 Dec;30(12):3672-9.
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