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The clinical importance of non-HLA specific antibodies in kidney transplantation

Akademisk avhandling

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av

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This thesis is based on the following papers:

I. Detection of complement-fixing and non-fixing antibodies specific for endothelial precursor cells and lymphocytes using flow cytometry. Ayeda AlMahri, Jan Holgersson, Mats Alheim. Tissue Antigens, 2012, 80, 404–415

II. The outcome of the endothelial precursor cell crossmatch test in lymphocyte crossmatch positive and negative patients evaluated for living donor kidney transplantation. Mats Alheim, Ayeda AlMahri, Jakob Nilsson, Gunnar Tydén, Jan Holgersson. Human Immunology, 2013, 74, 1437–1444

III. A pre-transplant positive endothelial precursor cell crossmatch does not imply reduced long-term kidney graft function. Markus Gäbel, Ayeda AlMahri, Lennart Rydberg, Jan Holgersson, Michael E. Breimer. (Manuscript).

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The clinical importance of non-HLA specific antibodies in kidney transplantation

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Abstract

The clinical significance of human leukocyte antigen (HLA) antibodies (Abs) for hyperacute, acute and chronic antibody-mediated rejection (AMR) of kidney allografts has been clearly demonstrated. AMR occurs in the absence of donor-reactive HLA Abs. It is not known how common the problem of AMR by non-HLA Abs is because of lack of suitable assays for their detection. It is believed that the non-HLA Ab population, although heterogenic, is likely to target antigens on donor organ endothelial cells (ECs). We have been involved in the clinical introduction of a flow cytometric (FC) crossmatch (XM) test that permits the detection of Abs reactive with endothelial precursor cells (EPC) isolated from donor peripheral blood. In this context the EPCs may function as surrogates for mature vascular ECs.

The work in this thesis describes the adaptation of the EPCXM to detection of complement-fixing HLA and non-HLA Abs using complement fragment-specific antibodies and flow cytometry, describes the outcome of the EPCXM in relation to the conventional lymphocyte XM (LXM), degree of HLA sensitization and transplantation outcome in patients evaluated for living donor (LD) kidney transplantation (Tx), and assesses the long-term renal graft function in patients with a positive EPCXM pre-transplant.

In the first paper, we investigated whether EPCs could be used for detection of complement-fixing Abs and if complement factor and IgG deposition on co-purified T and B cells correlated to the outcome of the T- and B-cell complement-dependent cytotoxicity (CDC) XM. Incubation of EPCs with HLA Ab-positive serum samples resulted in deposition of complement factors C3c and C3d, but not C1q nor C4d, on EPCs and co-purified lymphocytes. The amount of C3c deposition and IgG binding on EPCs and T cells, but not B cells, correlated. The specificity and sensitivity for C3d deposition on co-purified T cells *vs* the T CDC assay were 69% and 72%, while for B cells the sensitivity was considerably lower. In the second paper, we show that 32% of the LD patients had IgG and/or IgM-binding donor EPCs in their pre-Tx sera. Twenty-five percent of the patients were EPCXM IgM+. Of the patients with negative LXM tests, 25% had EPC Abs mainly of IgM class not reactive with HLA. There was no difference in rejection frequency or serum creatinine levels between the EPCXM positive and negative groups, which is in contrast to earlier published results. However, the clinical protocols used in the second paper included Ab pre-Tx treatments such as B cell depletion and Ab removal. The pre-Tx EPCXM positive group had significantly more patients with delayed graft function. In the manuscript we show that the difference in serum creatinine and glomerular filtration rates observed between EPCXM positive and negative groups at three and six months post-Tx disappears hereafter and during the four-year follow-up.

The detection of complement factors on EPCs and lymphocytes by flow cytometry allowing detection of complement-fixing non-HLA and HLA Abs widens the diagnostic repertoire that can be offered patients undergoing kidney transplantation and should thereby improve their clinical management. Prospective studies with appropriate control groups are needed to establish whether pre-treatments aiming at removing anti-EC Abs, as detected by the EPCXM pre-Tx, have a beneficial effect on short- and long-term graft survival.

Keywords: human leukocyte antigen, antibody-mediated rejection, endothelial precursor cells, complement-dependent cytotoxicity

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