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Mechanisms of liver allograft rejections

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Paper I: Are preformed antibodies to biliary epithelial cells of clinical importance in liver transplantation? Xupeng Ge, Bo-Göran Ericzon, Grzegorz Nowak, Henrik Öhrström, Ulrika Broomé and Suchitra Sumitran-Holgersson. Liver transplantation 2003;9(11):1191-8. Copyright © (2003), Wiley-Liss, Inc.

Paper II: Biliary epithelial cell antibodies induce expression of toll-like receptors 2 and 3: a mechanism for post-liver transplant cholangitis? Xupeng Ge, Mehmet Uzunel, Bo-Göran Ericzon, and Suchitra Sumitran-Holgersson. Liver transplantation, in press. Copyright © (2005), Wiley-Liss, Inc.

Paper III: A novel mechanism of liver allograft rejections facilitated by antibodies to liver sinusoidal endothelial cells. Suchitra Sumitran-Holgersson, Xupeng Ge, Bo Xu, Azza Karrar, Silvia Nava, Ulrika Broomé, Grzegorz Nowak, Bo-Göran Ericzon. Hepatology 2004;40(5): 1211-21. Copyright © (2004), Wiley-Liss, Inc.

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To Hongmei and Qingyue

ABSTRACT

Liver transplantation (LTx) has become a standard treatment for many end-stage liver diseases. However, acute rejections still occur in about half of the liver transplant recipients even treated with a combination of different modern immunosuppressants. The ultimate goal of liver transplantation is tolerance induction, which largely relies on the understanding of mechanisms involved in acute rejection process.

The exact mechanisms of liver allograft rejections are not yet elucidated and there is a great paucity of data regarding the role of humoral immunity. We were therefore interested in elucidating some of the mechanisms underlying liver allograft rejections. Specifically, we were interested in:

i) Detection and clinical correlation of the presence of antibodies in liver transplant patients to surface antigens expressed on the clinically relevant target cells of destruction and

ii) Determining the possible functional role of these antibodies in mediating liver allograft rejections.

iii) In addition, we also attempted to define the role of liver sinusoidal endothelial cells in allograft rejections in an experimental model.

We performed the following studies to evaluate the significance of antibodies to liver specific cells such as biliary epithelial cells (BEC) and liver sinusoidal endothelial cells (LSEC) in 95 liver transplant patients. In paper I, we found that preformed antibodies to BEC are associated with acute liver allograft rejections. In paper II, antibodies to BEC in the post-LTx period were found to be associated with cholangitis. Functional analysis of post-transplant antibodies to BEC showed they are capable of inducing toll-like receptor 2 and 3 as well as proinflammatory cytokine and chemokine expression. In paper III, the role of antibodies to LSEC in LTx was studied. Antibodies to LSEC significantly correlate to acute liver allograft rejections. We demonstrated that antibodies to LSEC may facilitate acute rejection episodes by regulating cellular immune responses. We further observed *in vivo* antibody deposition in liver biopsies taken from patients with antibodies to BEC or LSEC during acute rejection and cholangitis episodes. We found that majority of the antibodies to BEC and LSEC are not specific for human leukocyte antigens (HLA), and they do not cross react with other control cell types. Immunoglobulin classes of these antibodies are a mixture of IgG and IgM.

In paper IV, by using a fully MHC mismatched rat liver transplantation model, we were able to compare the difference between rejection and spontaneous acceptance (tolerance) of rat liver allografts. One of the earliest and most profound detectable differences between the two groups was the significantly decreased endocytic functional capacity of LSEC in the rejecting group.

Our findings indicate that presence of non-HLA antibodies to tissue specific antigens is a risk factor for rejections or post-transplant complications. Antibodies may contribute to liver allograft rejections by modulating cellular immune responses. B cell targeting therapy and strategies to help maintain LSEC functional integrity after transplantation may be beneficial for liver transplant patients.

LIST OF PUBLICATIONS

The thesis is based on the following papers:

I. **Xupeng Ge**, Bo-Göran Ericzon, Grzegorz Nowak, Henrik Öhrström, Ulrika Broomé and Suchitra Sumitran-Holgersson. Are preformed antibodies to biliary epithelial cells of clinical importance in liver transplantation? Liver transplantation 2003;9(11):1191-8

II. **Xupeng Ge**, Mehmet Uzunel, Bo-Göran Ericzon, and Suchitra Sumitran-Holgersson. Biliary epithelial cell antibodies induce expression of toll-like receptors 2 and 3: a mechanism for post-liver transplant cholangitis? Liver transplantation, in press

III. Suchitra Sumitran-Holgersson, **Xupeng Ge**, Bo Xu, Azza Karrar, Silvia Nava, Ulrika Broomé, Grzegorz Nowak, Bo-Göran Ericzon. A novel mechanism of liver allograft rejections facilitated by antibodies to liver sinusoidal endothelial cells. Hepatology 2004;40(5): 1211-21

IV. **Xupeng Ge**, Grzegorz Nowak, Bo-Göran Ericzon, and Suchitra Sumitran-Holgersson. A critical role for liver sinusoidal endothelial cells in spontaneous acceptance of rat liver allografts. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ADCC	antibody-dependent cell-mediated cytotoxicity
APC	antigen presenting cell
BEC	biliary epithelial cell
CDC	complement dependent cytotoxicity
CTLA-4	cytotoxic T lymphocyte-associated antigen 4
ELISA	enzyme-linked immunosorbent assay
ENA-78	epithelial neutrophil activating peptide-78
ERCP	endoscopic retrograde cholangiopancreatography
FACS	fluorescence activated cell sorter
FCXM	flow cytometric cross-match
HAEC	human aortic endothelial cell
HLA	human leukocyte antigen
ICAM-1	intercellular adhesion molecule-1
IFN-γ	interferon gamma
IL	interleukin
IQR	interquartile range
I/R	ischemia-reperfusion
LEC	lung epithelial cell
LSEC	liver sinusoidal endothelial cell
LTx	liver transplantation
MCC	mixed cell culture
MCP-1	monocyte chemoattractant protein-1
MHC	major histocompatibility complex
MIP-1α	macrophage inflammatory protein-1 alpha
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PRA	panel reactive antibody
PTC	percutaneous transhepatic cholangiography
RPTEC	renal proximal tubular epithelial cell
RT-PCR	reverse transcriptase-polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate/polyacrylamide gel electrophoresis
TCR	T cell receptor
TGF-β	transforming growth factor beta
TLR	toll-like receptor
TNF-α	tumor necrosis factor-alpha

1 Introduction

1.1 Organ transplantation

The idea to replace a diseased organ with a healthy one from another individual has been tested even centuries ago.[1] The primary obstacle people met at early stages is the surgical challenge. However, it was soon realized that there was a reaction between the recipient and the foreign organ, which would destroy the transplanted organ anyhow and was recognized as "rejections" later on. Alexis Carrel, whose description of vascular anastomosis is still being used today, concluded in 1914 that "the technical problems of transplantation were essentially solved, but until some method was developed to prevent the reaction of the organism against the foreign tissue, there would be no clinical application of organ transplantation".[2] The first successful kidney transplantation performed between identical twins in 1954 at the Peter Bent Birgham hospital in Boston clearly showed that renal failure could be reversed completely with kidney transplantation provided that rejections could be controlled in some way.[3] Enormously encouraged by the success of renal transplantation between identical twins, attempts of transplantation of other organs like liver, heart, and pancreas were followed using azathioprine, corticosteroids and anti-lymphocyte globulins as the major immunosuppressive drugs. It was not until 1980s that organ transplantation became a reliable treatment accepted widely by health professionals and patients. Introduction of cyclosporine in the early 1980s, a calcineurin inhibitor that dramatically reduced acute rejection episodes and improved graft survival, was a major step for modern transplantation. During the last decade, more immunosuppressive drugs like tacrolimus, mycophenolate mofetil and sirolimus became available. Nowadays the one-year graft survival rate for most types of organ transplantation approaches 80-90%.[2]

According to the relationship between donor and recipient, grafts can be divided into autograft, isograft, allograft and xenograft. Autograft is self-tissue or cells transferred from one body site to another in the same individual. Isograft is tissue or cells transferred between genetically identical individuals like identical twins. Allograft is tissue or cells transferred between genetically different members of the same species and xenograft is tissue or cells transferred between different species.[4]

1.1.1 Transplant rejections

There are three forms of allograft rejections, namely hyperacute, acute and chronic rejection. Hyperacute rejection occurs in about 0.5% of kidney transplant patients.[5] It is a rapid process that usually occurs within a few minutes or hours after transplantation. Occlusion of the vasculature following widespread endothelial cell destruction is the major histological feature. Presence of preformed HLA antibodies or ABO blood group antibodies are the major reason for hyperacute rejections.[6,7] Acute rejections usually take place within the first few weeks after transplantation. They are mainly mediated by cellular immunity with a feature of massive infiltration of macrophages and lymphocytes at the site of tissue destruction.[8] Chronic rejection can occur from months to years after transplantation. The mechanism mediating chronic rejections are less well

elucidated as compared with hyperacute and acute rejections. It is generally believed that both humoral and cellular immunity are responsible for chronic rejections.[9,10]

1.1.2 Challenges

Even though organ transplantations have become a standard treatment for many end-stage diseases, challenges still remain. First of all, acute rejections still occur in about 45-65% of liver transplant patients and 20-46% of kidney transplant patients even with a combination of several modern immunosuppressive drugs.[11-14] Meanwhile, immunosuppressive drugs have to be used all life long and most of them are non-specific, which as a consequence brings about increased incidence of infections, malignancy and other side effects like nephrotoxicity and neurotoxicity. Therefore, transplantation tolerance that is defined as long-term allograft survival in the absence of continuous immunosuppressive therapy is highly desired.[15] Another challenge is organ shortage, which contributes to the patient death on the waiting list. Approaches of solving the problem are, use of marginal donor, split organ, living related graft and xenotransplantation.

1.2 Liver transplantation and immunological characteristics

The first human liver transplantation (LTx) was performed in 1963.[16] Nowadays LTx has become the only curable therapy for many end stage liver diseases, including primary biliary cirrhosis, primary sclerosing cholangitis, hepatitis B and C, alcoholic liver cirrhosis, autoimmune hepatitis, metabolic liver diseases, hepatocellular carcinoma, and so on.[17] The general one-year survival of LTx has reached to 80%-90%.[18] From an immunological point of view, liver transplantation is different from other organ transplants in many ways. For instance, in certain rats, most mice and some pigs, liver allograft can be accepted spontaneously across a full MHC barrier.[19-21] Furthermore, spontaneous acceptance of liver allografts was accompanied with the development of immunologic tolerance toward various donor-originated organs.[19,22] In addition, it has been shown that in animal model administration of antigen via portal vein leads to immune tolerance and direct venous drainage of an allograft into portal system results in increased acceptance of the graft.[23,24] In human liver transplantation, hyperacute rejections rarely occur. HLA match and donor specific lymphocyte crossmatch are not normally considered to be important in liver transplantation.[25] All of these make liver an immuno-privileged organ. It is not fully understood why liver favors tolerance induction rather than the induction of immunity. A number of mechanisms have been suggested, including soluble HLA antigens produced by liver, passenger leukocytes, development of regulatory T cells,[26-28] as well as the role of liver sinusoidal endothelial cells.[29,30]

1.3 Liver transplant rejections

Hyperacute rejection caused by preformed antibodies is very rare in liver transplantation. Only few cases have been reported over the past 40 years.[31-33] It is well known that liver graft is resistant to hyperacute rejections even in the presence of a positive donor-specific crossmatch and panel reactive antibodies.[34] The exact

mechanism is not known, but the efficient clearance of circulating immune complexes mediated mainly by liver sinusoidal endothelial cells and Kupffer cells seems to play an important role.

Acute rejections occur in about 45% - 65% of liver transplant patients and usually within the first few weeks after transplantation.[11,12] Histopathological characteristics of acute rejection are lymphocyte infiltration of portal tracts associated with bile duct damage and inflammation of portal and hepatic venular endothelium. Cellular immunity, especially CD4+ and CD8+ T-cell mediated immune responses are believed to be the major mechanisms underlying acute liver allograft rejections.[35] Probably due to the belief that liver is more resistant for antibody-mediated rejections, so far there are few studies regarding the role of humoral immunity in acute liver allograft rejections as compared with kidney transplantation. Recently, some studies showed that C4d deposition is found in endothelia of portal vein and hepatic artery in portal area, and also found in the sinusoids of grafted liver.[36,37] These are important findings which indicate that cellular immunity may be not the only player during acute liver graft rejection episodes.

The incidence of late graft failure in liver transplantation is about 3-5% and chronic rejection is considered to be the primary cause.[9] The histopathological features of chronic rejection in liver graft is the presence of a foam cell arteriopathy and the loss of bile ducts in at least 50% of portal tracts.[38] The precise immunological mechanisms that cause chronic rejection are unknown. Both humoral and cellular immunity are believed to be responsible in this process.[9]

As we mentioned above, there are two basic mechanisms for allograft rejections, namely cellular rejection and humoral rejection.

1.4   Cellular basis of liver allograft rejections

1.4.1   Transplant antigens and HLA matching

Different individuals within the same species have different antigens expressed on their cell surface. The rejection of tissue transplanted between such individuals is the consequence of immune recognition of graft antigens by the host immune cells. Antigens are different in terms of their capacity for stimulating rejection response. The gene loci coding for antigens responsible for most vigorous allograft-rejection reactions are known as major histocompatibility complex (MHC). In human MHC is also called human leukocyte antigen (HLA) complex. Gene loci that are responsible for less vigorous rejections are known as minor histocompatibility gene. Often in kidney and routinely in bone marrow transplants one strives to find the best HLA match between donor and recipient to reduce the risk of allograft rejections.

Earlier HLA typing was usually accomplished by microcytotoxicity test. With the development of modern technologies it is now possible to do HLA typing by PCR.[39] It has been shown that HLA matching is important for kidney and bone marrow transplantation,[40,41] but less important in liver and heart transplantation.[42] So in clinical

practice HLA matching is not routinely performed in liver transplantation or the results are not considered in organ allocation. Rejection may still occur in HLA identical kidney transplants, indicating that minor histocompatibility antigens also contribute to allograft response. Since even HLA is not considered in LTx, the possibility that minor histocompatibility antigens play an important role in LTx is very low. Recent studies have shown that MHC class I chain-related antigen A and B (MICA and MICB) may play a role in renal allograft rejection as well,[43] but nothing is known about their role in LTx.

1.4.2 Antigen presentation and costimulation

Allorecognition starts by a process called antigen presentation. It occurs between antigen presenting cells and host T cells. The classic antigen presenting cells are dendritic cells, macrophages and B cells. Depending on the origin of antigen presenting cells (APC), allorecognition can be further divided into direct and indirect pathway. In direct pathway the antigen presenting cells are from donor origin, whereas in indirect pathway the antigen presenting cells are from the recipient. Both CD4+ and CD8+ T cells can be activated by antigen presenting cells, even though it is believed to be CD4+ T cells in most of the cases.[44]

The first signal for antigen presentation occurs when foreign peptide together with MHC molecules on APC binds to T cell receptors (TCR) on host T cells. The second signal for T cell activation is called costimulatory signal, which includes CD40:CD154, B7:CD28, and LFA-1:ICAM-1. (Fig.1) The third signal for T cell activation is provided by cytokines.[45] CD40 is constitutively expressed on antigen presenting cells while CD154 is transiently induced predominantly on CD4+ T cells.[46] CD154 is found expressed shortly after the interaction between foreign peptide and MHC complex and T cell receptor.[47] CD28 is constitutively expressed at high level on T cells, whereas B7 is usually absent (B7.1 or CD80) or at low and insufficient level (B7.2 or CD86) on antigen presenting cells. Activated antigen presenting cells can express B7.1 and upregulate B7.2 level upon stimulation.[48] Binding of CD40 to its ligand CD154 can induce or increase B7 expression on antigen presenting cells.[46]
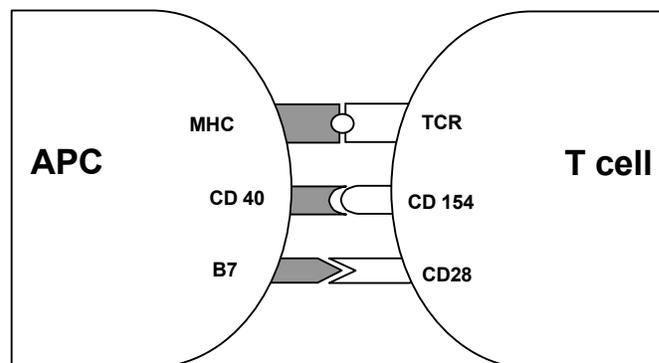

Figure 1. Antigen presentation and costimulatory signals

In liver transplantation both direct and indirect pathway are involved in allorecognition and direct pathway is believed to play a prevailing role at least early after transplantation.[35] The professional antigen presenting cells in the liver are bone marrow derived dendritic cell (also called passenger leukocyte). Studies showed that passenger leukocytes are released into circulation from liver graft and then migrate into the recipient spleen where they present antigens to host T cells and trigger T cell activation.[49] The significance of this direct activation decreases with time because the normal life span of passenger leukocytes in the graft and in the periphery is relatively short. Soluble or released MHC antigens from the graft can be processed by host antigen-presenting cells in regional lymph nodes and presented to recipient CD4+ T cells, which constitutes the indirect pathway of allorecognition.[50]

In addition to the professional antigen presenting cells, mouse liver sinusoidal endothelial cells (LSEC) express MHC class II, CD40 and CD80 and CD86. They have been shown to function as antigen presenting cells and present antigens to CD4+ and CD8+ T cells leading to tolerance instead of rejection.[51,52] Human LSEC express MHC class II and CD40, but not CD80 and CD86.[30] Less is known about the immunological functions of LSEC in human liver transplantation.

The knowledge acquired from costimulatory signals provides us a number of choices for tolerance induction via costimulation blockade. Cytotoxic T lymphocyte-associated antigen (CTLA-4/CD152) is a molecule expressed on activated T cells with a 20-fold higher affinity binding to B7, and this binding sends an inhibitory signal to T cells. A soluble fusion protein CTLA4-Ig consisting of CTLA-4 molecule and the constant region of IgG heavy chain is available to block the interaction between CD28 and B7 leading to T cell anergy. Another costimulation blockade approach is to use antibody against CD154. Both CTLA4-Ig and anti-CD154 monoclonal antibody have been studied individually, in combination, or with different immunosuppressive drugs in rodents and non-human primates with prolongation of various organ allografts.[53-57] Despite the promising results from preclinical experiments, the application of costimulation blockade in the clinic is disappointing. Humanized anti-CD154 monoclonal antibody has been used in a clinical trial composed of 7 renal transplant patients. Unfortunately it had to be discontinued due to the side effects of thromboembolic toxicity and high incidence of rejection episodes. Currently the only costimulation blockade reagent in clinical development is LFA29Y (a modified form of CTLA4-Ig), which is under evaluation in phase 2 clinical trial.[58] The discrepancy of results from animals and clinical trials may reflect that human immune system is more complex or the optimal use of these agents has to be reconsidered.

In liver transplantation costimulatory blockade has been tested only in some animal models. Bartlett AS, et al. used anti-CD154 monoclonal antibodies in DA to Lewis rat liver transplantation model resulting long time survival without signs of acute rejections.[59,60] CTLA4Ig was used in ACI to Lewis rat liver transplant model achieving indefinite survival of liver grafts and it has also been tried in mice resulting in prolongation of liver allograft survival.[61,62]

1.4.3 Cell-mediated effector response

Delayed-type hypersensitivity: In the sensitization stage, host CD4+ T helper cells are activated via interaction with antigen presenting cells. The origin of antigen presenting cells could be donor-derived (direct recognition) or recipient-derived (indirect recognition). Activated T helper cells start to proliferate and produce cytokines which induce three major effector mechanisms: i), the production of cytokines like IFN-γ is important for activating macrophages and promoting its influx in the graft. The subsequent activation of macrophages amplify the inflammation process by producing more cytokines, chemokines, proteolytic enzymes and other soluble factors; ii), IL-2 produced by activated T helper cells can promote the generation and proliferation of CD4+ and CD8+ cytotoxic T cells; iii), CD4+ T helper cells also promote B cell maturation and differentiation into plasma cells. The latter produce antibody and exert detrimental effect via antibody mediated effector mechanisms.[4,63]

Lymphocyte-mediated cytotoxicity: The CD8+ cytotoxic T lymphocytes can be primed and activated by direct presentation of donor passenger leukocytes from the graft. The destruction of target cells by CD8+ cytotoxic T lymphocytes involves perforin, granzymes and Fas-dependent apoptosis.[63,64]

So far not many studies can be found dealing with cellular effector mechanisms of liver allograft rejections,[65] probably because of the fact that cellular immune response has been well accepted to be the reason for acute liver graft rejection, so it should follow the common rules. Results from animal studies showed that the pattern of cell infiltration and cytokine profile in liver transplantation is quite similar to the picture of delayed-type hypersensitivity.[66] Pathological findings during acute human liver allograft rejections include intense portal infiltration with CD4+, CD8+ T cells and macrophages.[67] It was reported that cytotoxic T cell mediated cytotoxicity via granzyme B is one of the effector mechanisms during liver allograft rejection.[68]

1.4.4 Regulatory T cells

The idea of existence of suppressor T cell populations in the periphery was first described in the 1970s.[69] However, it was not until 1995 that a subpopulation of CD4+ T cells expressing the IL-2 receptor α-chain (CD25) was identified to be essential to control autoaggressive immune responses, which now is referred to as CD4+CD25+ regulatory T cells.[70] CD4+CD25+ regulatory T cells constitute about 5-15% of peripheral CD4+ T cells in human and mice.[71] It is well known that CD4+ T cells express CD25 after activation, but they are different from CD4+CD25+ regulatory T cells, because studies have shown that they are not suppressive.[70] Apparently only a subpopulation of the circulating CD4+CD25+ T cells is real regulatory cells. Recent study showed that the transcription factor Foxp3 seems to be a more specific marker for regulatory T cells.[72] CD4+CD25+ regulatory T cells is generally believed to arise within the thymus, then migrate to the periphery to exert their function,[73] but there is also evidence indicating that these cells could be generated in periphery as well.[71] IL-2 seems to play an important role in the development and maintenance of naturally occurring CD4+CD25+ regulatory T cells, because these cells are absent in IL-2 knock-out mice.[73] Naturally-occurring CD4+CD25+ regulatory T cells are crucial for regulating the development of autoimmune disease. Regulatory T cells can be also

induced after allograft transplantation when immunosuppressant or costimulation blockade is applied.[74,75] A recent study indicates the presence of "natural alloregulatory T cells" that are responsible for spontaneous acceptance of allografts in rodents, but whether this population is different from naturally occurring CD4+CD25+ regulatory T cells or not is unclear due to the lack of additional identification markers.[76,77]

The mechanisms by which CD4+CD25+ regulatory T cells exert suppression are not fully understood. Accumulating evidence indicates that CD4+CD25+ regulatory T cells require stimulation via T cell receptor to function and the suppression effects seem to be in a cell-contact dependent manner or trough short range signaling.[78,79] The target cells that regulatory T cells exert their suppression function on are responding T cells or antigen presenting cells. CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) that is expressed on the surface of regulatory T cells, IL-10 and TGF-beta seem to be involved in the regulatory T cell mediated suppression, but results from different groups are still controversial.[71,79] The site where regulatory T cells act to prevent allograft rejection are generally considered to be within the graft itself and the secondary lymphoid tissue.[79]

In organ transplantation, tolerance induced by costimulation blockade[57,75,80] and some immunosuppressive drugs[74,81] is believed to be due to the development of regulatory T cells. Therefore, there is an apparent attraction and excitement about the possibility of using these regulatory T cells as a therapeutic approach to induce tolerance in clinical transplantation. A few points still remain that limits its clinical application. For example, the frequency of these regulatory T cells in circulation is very low and they are refractory to stimulation *in vitro* due to their anergic nature, so it is difficult to get sufficient number of cells to treat ongoing immune responses. There are other challenges as well like the risks of uncontrolled proliferation and development of unforeseen functional activities due to the lack of knowledge about these cells.[82] All of these constitute the internal drive for the intensive studies in this field and hopefully this somatic-cell therapy will come into practice in the near future.

The existence of regulatory T cells has been suggested in Lewis to DA rat liver transplantation model by using adoptive transfer of spleen cells from tolerance animal to naive rats, but characterization of this cell population by cell markers has not been performed.[28,83] A recent study performed in 14 living donor liver transplant recipients who have stopped immunosuppression and developed operational tolerance showed that CD4+CD25+ regulatory T cells is one of the important factors maintaining liver graft survival in the absence of immunosuppression.[84]

1.4.5 Immunosuppression

The immunosuppressive drugs used today in the clinic are usually non-specific and affect mainly cellular immune responses. The major immunosuppressants and their action are listed as following:

Cyclosporin A, tacrolimus (FK506) and sirolimus (Rapamycin): Cyclosporin A and tacrolimus are calcineurin inhibitors, which can block the activation of resting T cells by inhibiting the transcription of genes encoding IL-2 and IL-2 receptors. Sirolimus can

block the proliferation and differentiation of activated T helper cells by binding to an immunophilin.[85,86]

Antimetabolites (azathioprine, mycophenolate mofetil): Azathioprine is a potent mitotic inhibitor that can reduce T cell proliferation by blocking the synthesis of inosinic acid in the S phase of cell cycle. Mycophenolate mofetil can inhibit inosine monophosphate dehydrogenase (IMPDH), the branch point of purine biosynthesis. Therefore, T-cell and B-cell proliferative responses and antigen-specific antibody responses are inhibited.[87,88]

Corticosteroids: Corticosteroids are potent anti-inflammatory agents and exert their effect at many levels of the immune response.[4]

Polyclonal antibodies: ATG (antithymocyte globulin) is a polyclonal antibody prepared by immunizing animals like horses with human thymocytes. The administration of ATG will result in the decrease of patient's total lymphocyte count due to complement and cell mediated lymphocyte depletion.[89]

Monoclonal antibodies: Anti-IL-2 receptor monoclonal antibodies (basiliximab and daclizumab) bind to the alpha chain of IL-2 receptor (CD25) that is expressed on activated lymphocytes. Monoclonal antibody against CD3 (OKT$_3$) molecule can rapidly deplete T cells from circulation. Use of anti-CD 20 antibody results in rapid decrease of B cells and circulating antibodies.[90-92]

The immunosuppression protocol used in liver transplantation was derived historically from those in kidney transplantation. At present, the most common protocol for liver transplantation is triple immunosuppression with tacrolimus or Cyclosporin A, mycophenolate mofetil or azathioprine, and steroid. Other combinations with sirolimus or new immunosuppressants like FTY720 are still mainly in clinical trial.[89,93] A recent study showed that calcineurin inhibitors (tacrolimus or Cyclosporin A) decrease CD4+CD25+ regulatory T cells in peripheral blood in liver transplant patients, which is a effect against tolerance induction.[94]

1.5   Humoral basis of liver allograft rejections

Traditionally, humoral immunity is considered to be mainly responsible for hyperacute rejection and part of chronic rejection.[5] More and more evidence demonstrates that humoral immunity also contributes to acute allograft rejections.[95] Nowadays, antibody-mediated rejection is believed to play a role in about one third of acute rejection episodes in renal transplantation.[96] It is generally believed that liver is resistant to antibody mediated rejections.[97] Very little has been known about the role of antibodies in acute liver allograft rejection.

1.5.1   General function of antibodies

Antibodies, the functional name of immunoglobulins, are produced by plasma cells and serve as effectors of humoral immunity. Immunoglobulins can be classified into five main isotypes according to their structure, namely IgA, IgD, Ig E, IgG and IgM (Fig.

2). IgA antibodies are found as two subclasses (IgA1 and IgA2), whereas IgG antibodies can be further subdivided into four subclasses (IgG1, IgG2, IgG3 and IgG4) in the order of their abundance in serum. IgM forms pentamers in serum, while secreted IgA appear either as monomer or as a dimmer.[4] The basic functions of antibodies are summarized as follows:

Complement activation: Antibodies can initiate complement activation by the classical pathway. The first component of classical pathway of complement activation is C1q, followed by a cascade of proteolytic cleavage reactions. Both IgG (IgG1 and IgG3) and IgM isotype can activate complement. Due to the structural difference, only one IgM molecule is enough to trigger complement cascade whereas at least two IgG molecules are needed. Complement receptor of immunoglobulins is important in the removal of immune complexes from the circulation.[4,98]
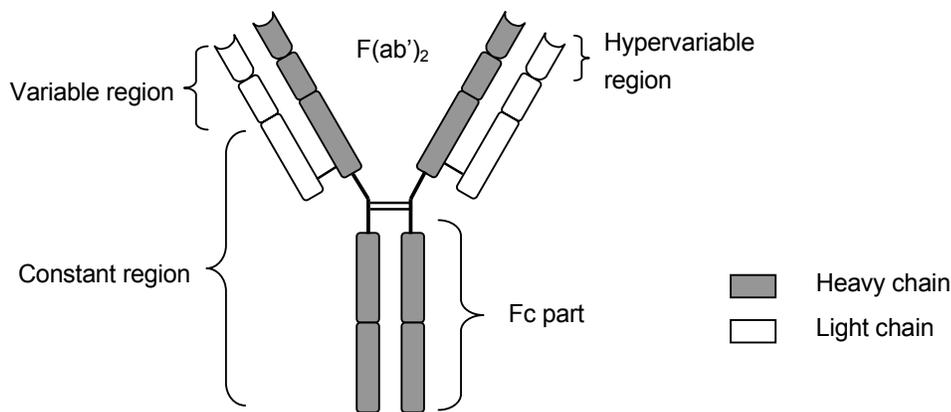

Figure 2. Typical structure of antibody molecule

Antibody-dependent cell-mediated cytotoxicity (ADCC): Target cell bound antibodies can link to NK cell and macrophages via Fc receptors. In this way the target cell can be killed by NK cell or macrophages by apoptosis.[4,98]

Opsonization: Opsonization refers to the promotion of phagocytosis of antigens by macrophages and neutrophils. Interaction between IgG antibody and Fc receptor, which are present on the surface of macrophages and neutrophils will promote the phagocytosis of antigens.[4] In allorecognition, antigen presenting cells internalize the alloantigens, then process and present to the recipient T cells. With the presence of antibodies, the uptake of alloantigens by antigen presenting cells will become more efficient, which was considered to be an indirect pathway of T cell activation facilitated by antibodies.[99]

Immunomodulatory function: Antibody upon binding may activate, inhibit or alter certain functions of the target cells. It has been shown that HLA class I antibodies may activate or inhibit T cell functions depending on the experimental settings used.[100-102]

1.5.2 T and B lymphocyte crossmatch

Prior to organ transplantation a crossmatch is usually performed to detect the presence of donor-reactive antibodies in the recipient. The traditional method is complement dependent cytotoxicity (CDC),[103,104] in which donor lymphocytes were incubated with recipient serum followed by complement addition and determination of cell killing. This method is good for detecting high level, complement fixing antibodies. With the development of modern technologies, more sensitive crossmatching methods like flow cytometric crossmatch has been developed with an advantage to detect low level complement fixing and non-complement fixing donor-reactive antibodies.[105] The antibodies detected by these methods were generally believed to be HLA antibodies. For donor and recipient lymphocyte crossmatch one can use unseparated lymphocytes as the target cells, but more commonly separated T and B cells are used. T cells express HLA class I molecules, as most of other tissues do. Therefore, positive T cell crossmatch is believed to be more important in organ allocation. B cells express both HLA class I and II molecules. The significance of B cell crossmatch has been controversial for many years probably due to the fact that positive reaction could be caused by a mixture of HLA class I and II (and perhaps non-HLA) antibodies.[106,107] By using a combination of T and B cell crossmatch, it might give some information about the HLA class I or II specificity of donor-reactive antibodies. A recent study performed by United Network for Organ Sharing (UNOS) using data from 9031 patients showed that a positive B cell crossmatch and negative T cell cross match is associated with poor outcome in kidney transplantation.[108]

One should keep in mind that the specificity of donor-specific antibodies detected by various T and B lymphocyte crossmatch methods is believed to be HLA antibodies, but this may not necessarily be true. So far, there is no well accepted number showing to what extent HLA antibodies and non-HLA antibodies contribute to a positive T and B crossmatch. For instance, non-HLA antibodies can be responsible for 2%-77% of positive B cell crossmatches.[109,110] The big discrepancy between different reports could be due to the difference in patient population, patient number, as well as the methods used.

Panel reactive antibodies (PRA) are detected by taking sera from potential transplant recipients and then incubating with a number of different lymphocytes of known HLA specificities. The purpose of this test is to assess the immunization status of a potential graft recipient and it can also help to predict the risk of giving a positive crossmatch against a potential organ donor.[111]

Pre-transplant crossmatch by various methods have greatly contributed to eliminate the occurrence of hyperacute rejections especially in kidney transplantation over the past decades.[6] However, liver transplantation are routinely performed in crossmatch positive patients without suffering the penalty of hyperacute rejection.[97] Only few cases of hyperacute liver rejections have been reported relating to the presence of strong positive donor-specific crossmatch.[31,112] Earlier results showed that there is no correlation between a positive crossmatch and acute rejection episodes in liver transplantation.[113-115] Later, several studies showed that a positive donor-reactive lymphocyte crossmatch is associated early acute rejection episodes and graft loss.[116-118]

The long-term impact of positive crossmatch on chronic liver rejections is still controversial. Some studies showed that neither panel reactive nor donor specific HLA antibodies are associated with chronic liver allograft rejections,[115,119,120] but others showed that a positive crossmatch is related to vanishing bile duct syndrome.[112,121] The discrepancy between these results could be due to the number of patients included, time of follow-up, the immunosuppression protocol applied, and the method used for crossmatching. Apparently, the role of crossmatch in chronic liver transplant rejections needs to be explored by further studies.

1.5.3 Alloantibodies

Alloantibodies are produced as a result of allo-recognition and can be divided into HLA antibodies and non-HLA antibodies.

HLA antibodies:

Donor-specific HLA antibodies are considered to be very important in organ transplantation and many studies have confirmed that HLA antibodies are directly related with hyperacute rejections and also associated with acute rejection episodes and poor graft outcome.[7,122,123] Non donor-specific HLA antibodies, characterized by a negative donor lymphocyte crossmatch and presence of panel reactive HLA antibodies, was first reported by Terasaki, et al. in 1971 to be associated with decreased graft survival.[124] Later, more studies have confirmed this finding,[125,126] even though good outcomes are not uncommon in hyperimmunized patients.[127] It is hard to say which specificity of HLA antibodies is more important in organ transplantation due to the fact that routine crossmatch methods used cannot safely distinguish between HLA class I and II antibodies. Recently, antibodies to nonclassical MHC class I-related chain A (MICA) and B (MICB) have been shown to be correlated with acute rejection and poor graft survival in renal transplantation.[128]

HLA antibodies detected by a positive crossmatch are related to a few cases of hyperacute liver rejections.[31,129,130] Some studies showed that HLA antibodies have no effect on acute liver allograft rejections, but results from recent studies showed the contrary.[113-118] The effect of HLA antibodies in chronic liver rejections is still controversial. [112,115,119-121]

Non-HLA antibodies:

Blood group antibodies presented in ABO-incompatible transplantation is associated with hyperacute rejections and usually considered to be a contraindication of transplantation.[131,132] Even though only a few cases of hyperacute rejection in ABO-incompatible liver transplants have been reported over the years, the general long-term result is poor.[130,131,133] Recently, due to the development of B cell targeting therapy and the shortage of available organs, ABO-incompatible living donor renal transplant has been performed with promising results.[134,135] Several cases of liver transplantation with ABO-incompatible grafts were also reported using various B cell targeting therapy.[136-138]

Non-HLA endothelial cell antibodies were found to play a role in allograft rejections when kidney transplants performed between HLA-identical siblings were rejected vigorously.[139] Activated graft endothelium express HLA class I and class II antigens, so it may be the target of preformed HLA antibodies.[140] While, the presence of these HLA antibodies can be detected by routine crossmatch. A number of other non-HLA antigenic determinants are expressed on endothelial cells as well. It has been shown in crossmatch negative transplants that endothelial cell antibodies are of major importance in kidney and heart transplantation.[141-146] It is estimated that approximately 10% of C4d positive acute renal allograft rejections is due to non-HLA antibodies.[147] It has been difficult to perform a routine donor-specific endothelial cell crossmatch. A recent study described a quick and easy method to isolate precursor endothelial cells from peripheral blood, which provides a promising approach for endothelial cell crossmatch.[148] There is a paucity of information on the role of endothelial cell antibodies in liver transplantation before we published our paper on liver sinusoidal endothelial cell antibodies that is also included in this thesis.[149]

There are scattered reports regarding the significance of other non-HLA antibodies in transplantation. For example, monocyte antibodies were considered to be associated with severe rejections and renal graft failure.[150] The presence of antibodies to epithelial cells was found to be associated with decreased graft survival and post-transplant infections in lung transplantation.[151] However, no information is available regarding the role of these non-HLA antibodies in liver transplantation.

1.5.4 Autoantibodies

Autoantibodies are believed to be a result of immune recognition against "self" antigens via mechanisms that are still not fully understood. They include anti-nuclear, anti-nucleoprotein, anti-DNA, anti-cytoplasmic antibodies and many others. Autoantibodies are usually not pathogenic and mainly used as a diagnostic marker for different diseases. Compared with alloantibodies, especially HLA antibodies, autoantibodies have not been extensively studied in organ transplantation. However, there is some evidence showing that autoantibodies may also play a role in transplant rejections.[152-155]

Autoantibodies against blood cells can be pathogenic causing direct destruction of target cells,[156] but whether other autoantibodies are pathogenic is still controversial. Based on the clinical correlation between autoantibodies and diseases, a pathogenic role for autoantibodies is frequently suggested without direct evidence.[157] Recently, *in vivo* evidence in which antineutrophil cytoplasmic autoantibodies were transferred into mice leading to glomerulonephritis and vasculitis was provided, indicating that autoantibodies against targets other than blood cells can be pathogenic as well.[158] In organ transplantation, a recent study reported that autoantibodies against angiotensin II type 1 (AT_1) receptor may contribute to acute humoral rejections in renal transplant patients by activating AT_1 receptor and mimicking the action of angiotensin II.[152] It has also been reported that antivimentin antibodies are associated with chronic rejections in heart transplantations, and it was speculated that autoantibodies may cause graft damage in the similar manner as alloantibodies.[153]

In some of the liver transplant patients like primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis, autoantibodies can be detected because of their nature of autoimmune liver diseases.[159] Alcoholic liver disease is associated with autoimmunity, so it is possible to find autoantibodies in these patient groups.[160] Most of these antibodies have been speculated to contribute to the recurrence of primary diseases, but little is known about their impact on liver transplant rejections.[161,162] Dubel L, et al. reported that post-transplant anti-tissue antibodies (against the smooth muscle or the nucleus) were strongly associated with chronic rejections in liver transplantation and suggested that these antibodies could be used to identify patients at high risk of developing chronic rejection after liver transplantation.[154] It was also reported that the appearance of anti-smooth muscle antibodies (SMA) are associated with acute liver graft rejections in autoimmune hepatitis patients. They speculated that the production of these antibodies was due to the release of intracellular autoantigens by liver injury following rejection.[155]

1.5.5 Effector mechanisms in antibody mediated rejections

In general, effector mechanisms involved in antibody mediated rejections include: i), activating complement cascade; ii), endothelial cell activation and triggering coagulation cascade; iii), ADCC with antibody Fc part binding to NK cells and macrophages promoting the killing of target cells;[63] iv), the binding of antibodies on target cells in the graft might attract Fc receptor-bearing inflammatory cells to the graft, thereby contributing to inflammation response during rejections episodes;[6] v), during indirect allorecognition process, graft-derived alloantigens are taken up by host antigen presenting cells, processed and presented to the recipient T cells. The uptake of alloantigens is greatly enhanced by the presence of specific antibodies. In this way antibodies may facilitate alloimmune response.[6,99] vi), In addition to these classic mechanisms, a regulatory role of non-cytotoxic antibodies may also be envisaged. It has been shown that HLA class I antibodies may enhance T cell proliferation upon binding.[100,101] It has also been reported recently that anti-HLA class I antibodies could activate endothelial cells and promote chronic rejection.[163]

In hyperacute rejection, preformed antibodies bind to blood group ABO or MHC antigens expressed on the surface of endothelial cells activating complement and coagulation cascades. As a result, hyperacute rejection is featured by thrombosis of small vessels by platelet thrombi, interstitial hemorrhage and severe endothelium injury.[63] Hyperacute rejections in liver transplantation follow these mechanisms.[130,131]

Acute rejection is generally accepted as a T-cell-mediated process. However, repeated clinical observations implicated that antibodies may play a central role at least in a subset of patients with acute rejections. Acute humoral rejection was mainly identified in kidney transplant patients with histological characteristics involving infiltration of peritubular capillaries and glomeruli by neutrophils and monocytes, capillary fibrin thrombi, sometimes fibrin necrosis and vasculitis, and deposition of complement split product C4d along peritubular capillaries. Therefore, complement activation is believed to be the major mechanism involved in acute humoral renal rejections.[164] In addition, it has been reported that antibodies binding to graft endothelial cells can attack target cells via ADCC with Fc binding to macrophages and NK cells.[165]

Very little is known about the role of antibodies in acute liver allograft rejection. Recently three papers from two centers were published showing that C4d staining can be found in some of the liver transplant patients undergoing acute rejection episodes, suggesting the involvement of antibodies in acute liver allograft rejections and possibly via complement activating pathway.[36,37,166] However, the number of patients included in these studies is very few.

At present, less is known about the pathogenesis of chronic rejections and most of our knowledge comes from animal experiments. Some studies showed that anti-HLA antibodies are correlated with chronic rejections in kidney and lung transplant patients, but it is difficult to infer causality based on these oberservations.[167,168]

1.5.6 C4d staining

Complement system is a complex system composed of more than 30 serum and cell surface components.[169] After organ transplantation circulating antibodies can bind to the graft endothelial cells and then trigger complement activation cascade. Since the bloodstream continuously clears the endothelial surface, the ideal candidate to be used as a maker of antibody mediated response should have stable binding to the endothelia of the allograft. C4d is an inactive fragment of complement C4 released during the activation of classical pathway and particularly privileged because of its covalent binding to graft endothelia.[170] After its first description in 1991,[171] peritubular capillary complement C4d staining in graft biopsies has been widely accepted as a unique marker of antibody-mediated rejections. About 25-50% of all biopsy-confirmed acute renal allograft rejection episodes are C4d positive, indicating the presence of acute humoral rejections.[147] A recent study showed that 95% of allograft biopsies from recipients with donor-specific alloantibodies at the time of rejection had positive C4d staining.[172] Therefore, classification of renal allograft rejections have been revised accordingly with a supplementation of criteria for antibody mediated rejection.[173]

The role of C4d staining in chronic renal transplant rejections is still controversial. Mauiyyedi et al. reported that 61% of biopsy samples from patients with chronic allograft nephropathy are positive for C4d staining,[174] while positive rates of C4d staining in others' reports are much lower (13-34%).[175,176] Nickeleit et al. suggested that C4d staining is a marker for acute but not chronic humoral rejection.[177] It is quite clear that more studies are needed to elucidate the role of C4d as a marker of humoral response in chronic rejections.

The significance of C4d staining in other organ transplants remains to be further explored. Preliminary results suggest that C4d could play a similar role in heart transplantation as in kidney transplantion.[178,179] In some of the acute liver allograft rejections, C4d deposition was found in endothelia of portal vein and hepatic artery in portal area, and was also found in the sinusoids.[36,37,166] However, one should bear in mind that liver sinusoidal endothelial cells are capable of taking up immune complexes,[180] so the relevance of positive C4d staining in the liver sinusoids need to be further defined. There is also evidence showing that alternative complement activation

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pathway may also be involved in liver transplantation.[181] Taken together, more studies are needed to elucidate the role of C4d staining in liver transplantation.

1.5.7   B-cell targeting therapies

The overall outcome of acute humoral rejection is considered to be worse than acute cellular rejection.[147,182] Therefore, therapies targeting B cells and humoral immunity have received a great deal of interest in the transplantation field. Currently, there are a number of B-cell targeting therapies available:

Intravenous immunoglobulin (IVIg) is IgG fraction prepared from pooled plasma from thousands of healthy donors. It contains 97% of IgG whole molecules, very little F(ab')$_2$ fragments and only traces of IgM and IgA. The immunological mechanisms for IVIg function are quite diverse, among which some may be particularly relevant to transplantation: i) neutralization of circulating antibodies via idiotype-anti-idiotype interactions; ii) inhibition of complement binding; iii) inhibition of B and T cell proliferation and activation.[183,184]

Immunoadsorption with protein A and plasmapheresis are strategies to remove circulating antibodies. It has been shown that in highly sensitized patients antibody titers dropped significantly with a couple of sessions of immunoadsorption and most of the patients could be transplanted.[185] Studies also showed that immunoadsorption and plasmapheresis can be used for the treatment of acute humoral rejections.[186,187]

Anti-CD20 antibody Rituximab is a chimeric monoclonal antibody. It has been used as an induction therapy for ABO incompatible transplant and highly sensitized patients as well as treatment of acute humoral rejections.[135,188,189] With only a single or a couple of doses of Rituximab, circulating antibody titers were reduced significantly.[135,190] The effects of anti-CD20 antibodies were believed to be due to the inhibition of B cell proliferation, induction of B cell apoptosis by ADCC and complement-dependent cellular cytotoxicity.[92]

Some immunosuppressive drugs also have some effects on B cells and humoral immunity. Studies have reported that mycophenolate mofetil can inhibit antibody production and humoral responses[191,192] and can limit B cell responses in renal allograft recipients with acute humoral rejection when used in combination with tacrolimus.[193]

There are some other B cell targeting strategies like splenectomy and cyclophosphamide. It is noteworthy that usually these B-cell targeting strategies were used together in different combinations to get better results.[135,194]

Many of the B cell targeting therapies is a result of modern technological development and the attention paid to them is greatly stimulated by the recent promising results from blood group ABO-incompatible living donor renal transplantations.[134,135] Some clinical trials with ABO-incompatible liver transplantation were also performed using various B cell targeting therapies like anti-CD20 monoclonal antibodies, IVIg, plasmapheresis, and spleenectomy.[136-138] However, the long-term results need to be closely monitored.

1.5.8　Mechanisms of liver allograft resistance to hyperacute rejections

It is well known that liver is rather resistant to antibody-mediated hyperacute rejections compared to other organs, but the exact mechanisms are still not clear. A number of immunological and physiological factors have been suggested to be involved. First of all, liver has strong clearance function. Formation of immune complexes with antibodies binding to soluble HLA class I antigens shed from the liver can be efficiently cleared away by the phagocytic function of Kupffer cell and liver sinusoidal endothelial cells.[195,196] Antibody clearance can also be enhanced by anti-idiotype antibodies.[197] The large volume of blood replacement during liver transplant surgery will dilute circulating antibodies and reduce the number of host B lymphocytes.[97] In addition, poor complement and platelet function associated with end-stage liver diseases may also contribute to the liver resistance to humoral insult.[198] Physiological factors like big liver mass and dual blood supply may also play a role.[199] In xenotransplantation and ABO-incompatible kidney allo-transplantations, sometimes grafts can survive in the presence of xeno- or allo-antibodies, a phenomenon termed accommodation.[200] Studies showed that endothelial cells in the accommodating grafts express products of surviving genes and complement regulatory proteins.[201,202] So far, little is known about how much this mechanism contributes to liver's resistance to antibody mediated rejections.

1.6　Biliary complications after LTx

Biliary complications after liver transplantation include bile leakage, bile duct strictures and infections. Bile leakage from the anastomotic site is usually technically related or due to bile duct ischemia. Surgery may be needed in the case of bile leakage.[203] Surgical bile duct strictures that occur at the anastomosis site usually can be identified by percutaneous transhepatic cholangiogram (PTC) or endoscopic retrograde cholangiography (ERC) and treated with surgical or radiological intervention.[203] The reason for non-surgical bile duct stricture is not very clear to date. Ischemia and immunological factors are believed to play a major role.[204,205] Bile duct infection (cholangitis) is not uncommon after liver transplantation.[206] Microbial infection, increased internal pressure as well as the manner of bile duct reconstruction are factors believed to be responsible for cholangitis episodes.

1.7　Biliary epithelial cells

Biliary epithelial cells (BEC) lining the biliary tract represent about 3-5% of the total cells in a normal liver.[207] The basic physiological function of BEC is to form and secrete bile and also transport IgA into the bile.[208] Biliary epithelium is one of the major targets during liver allograft rejections and a number of autoimmune liver diseases like primary sclerosing cholangitis and primary biliary cirrhosis.[209] However, there are not many studies regarding the immunological function of BEC during allograft immune response as compared with other cell types like endothelial cells, probably due to the difficulty in isolating and culturing the cells. One of the interesting and controversial questions is whether BEC can function as antigen presenting cells. BEC constitutively express HLA class I but not HLA class II molecules, while during inflammation enhanced HLA class I and induced HLA class II are found.[210] However,

BEC do not express the costimulatory molecules CD40, CD80 and CD86 either at mRNA or protein level even after cytokine stimulation, meaning that they may not be antigen presenting cells.[211] Nevertheless, some *in vitro* experiments showed that BEC are able to provoke allogenic proliferative immune response of naïve T cells, suggesting that BEC may have the potential to function as a semi-professional antigen presenting cells via other pathways, but there is no *in vivo* evidence so far.[212,213] What we have discussed above is the possible role of BEC in triggering immune responses. There is also evidence showing that BEC may facilitate the inflammation process by various mechanisms. For instance, expression of intercellular adhesion molecule-1 (ICAM-1), a molecule that is important for the adhesion of lymphocytes, was found on BEC during liver transplant rejection, infection, and in primary biliary cirrhosis.[214,215] Results from our group showed that in primary sclerosing cholangitis patients BEC express CD44 and produce high level of IL-6 upon autoantibody binding.[216] IL-6 is a well-known proinflammatory cytokine and CD44 is an adhesion molecule that can facilitate T cell recruitment.[217] It has also been reported the BEC can secrete chemokine IL-8 and MCP-1 (monocyte chemotactic protein-1), which promotes the recruitment of neutrophils, monocytes and T cells.[208]

In the case of autoimmune liver diseases, the role of cell-mediated immunity in pathogenesis is still not well established even though T cell infiltration is frequently found within the portal tract. Therefore, antibody-dependent cell-mediated cytotoxicity (ADCC) involving autoantibodies against biliary epithelium has been suggested.[218] In acute liver allograft rejections, intrahepatic bile duct injury and infiltration with inflammatory cells are one of the three criterias to establish the diagnosis.[67] The exact mechanisms by which BEC are injured during liver allograft rejection is not fully understood. The histological findings during acute rejection episodes strongly indicate that biliary epithelial damage may be the direct result of cellular effector response. Study showed that ICAM-1 expression on BEC facilitates the binding of cytotoxic lymphocytes and is important for cell-mediated cytotoxicity.[211] Constitutive expression of LFA-3 (lymphocyte-associated antigen-1) was found on BEC indicating that its interaction with CD2 on cytotoxic T cells and NK cells may lead directly to cytotoxicity.[219]

1.8   Liver sinusoidal endothelial cells

Strategic position and morphological characteristics: Liver sinusoidal endothelial cells (LSEC) represent about 60% of the non-parenchymal cells of the liver. They are strategically placed lining the hepatic sinusoid, therefore become the first contact of graft tissue with recipient lymphocytes (Fig. 3).[220] The lumen of liver sinusoid is narrow (about 5-7 µm). T cells pass the liver several hundred times per day and the flow is slow, which allows a full contact and interaction with LSEC.[221] LSEC possess fenestrations with a mean diameter of about 100 nm and without basement membrane.[222] It is thought that the exchange of fluid, solutes and particles between the blood and space of Disse takes place through these open fenestrae. However, experiments have shown that exogenous particles as small as 15 nm cannot pass freely through the fenestrae of LSEC to reach hepatocytes, indicating that LSEC are not just a physical separation between hepatocytes and blood stream but a functional barrier.[223]

Clearance function: LSEC are well known for their scavenger function that clears away physiological waste products as well as foreign particles. The clearance function of LSEC is mainly mediated by four groups of endocytic receptors, namely mannose receptor, hyaluronan/scavenger receptor, collagen-α chain receptor and Fc-γ receptor. The waste macromolecules that were cleared by LSEC are most types of connective tissue molecules, extracellular enzymes, lysosomal enzymes, soluble IgG-immune complex, and so on.[224] LSEC also remove away foreign material absorbed from intestinal tract, including virus, lipopolysaccharide and other pathogenic substances and foreign antigens, which constitutes an important part of innate immune system.[225] It is reasonable to assume that impaired endocytic function of LSEC due to different reasons of injury, which reflects a compromise of innate liver immunity, could have profound impact in liver transplant rejections.[226,227]
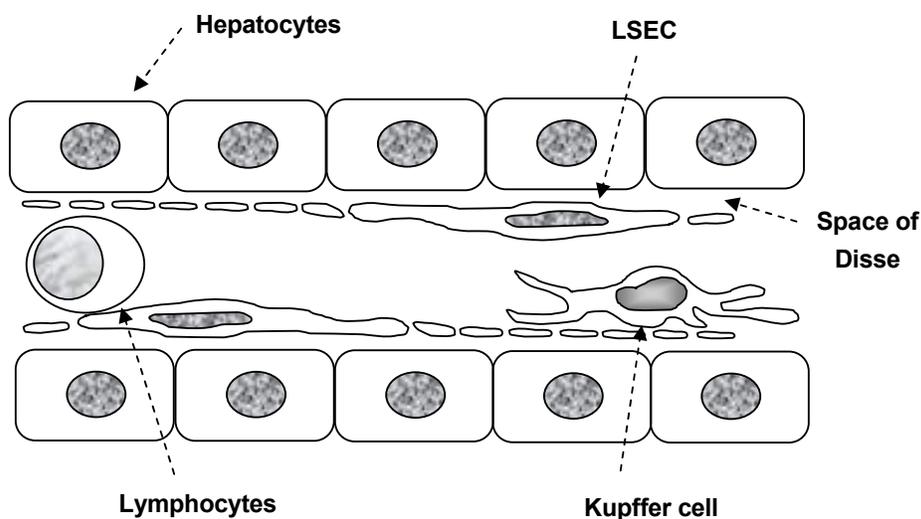
Figure 3. Structure of liver sinusoid.

Immunological function: LSEC are different from vascular endothelial cells in terms of phenotypic markers and functional capability (Table 1). Studies showed that mouse LSEC can function as antigen presenting cells and stimulate naïve CD4+ T cells. However, this priming fails to differentiate CD4+ T cells into T helper cells, instead they become regulatory T cells.[51] Mouse LSEC also have the capacity to present exogenous antigens on MHC class I molecules to CD8+ T cells, which leads to antigen-specific tolerance demonstrated by failure of CD8+ T cells to develop into cytotoxic T cells.[52] It is suggested that LSEC may prevent mounting immunity by rendering circulating T cells tolerant before they meet professional antigen presenting cells.[30] Because all hepatocytes are surrounded by LSEC, it is assumed that LSEC can protect hepatocytes from inadvertent immune attack by a combination of barrier and tolerogenic effect.[30] Liver is a place where activated and apoptotic CD8+ T cells accumulate during the clearance phase of peripheral immune response. Studies showed that liver can trap CD8+ T cells activated in the periphery and induce apoptosis.[228,229] It is not fully understood which cell type in the liver can induce T cell apoptosis.

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Unpublished results from our group show that LSEC induce T cell apoptosis via a TGF pathway.

Table 1. Phenotypic difference between human liver sinusoidal endothelial cells (LSEC) and human umbilical vein endothelial cells (HUVEC).[30,230]

Surface marker	LSEC	HUVEC
CD62E	-*	+*
CD31	-	++
FVIIIRAg	-	++
ICAM-1	++	++
VCAM-1	++*	++*
HLA class I	++	+
HLA class II	+*	+*
CD40	+	+
CD80	- (+ in mouse)	-
CD86	- (+ in mouse)	-

* Detected only after IFN-γ and TNF-α activation.

1.9 Toll-like receptors

Immune system can be classified as innate immunity and adaptive immunity. The function of the innate immune system is to provide a rapid protection after an infectious challenge and is usually thought to be non-specific. Adaptive immunity is mediated by clonally distributed B and T lymphocytes and characterized by specificity and memory.[231] The fruit fly, Drosophila melanogaster, doesn't have adaptive immune system. Therefore, it relies entirely on innate immunity to defend against microbial infection. In Drosophila, Toll is a type I transmembrane receptor, initially identified to be responsible for the establishment of dorso-ventral polarity in the developing embryo. However, later studies showed that they are crucial for the recognition of microbes by the innate-immune system.[232] This finding also challenged the dogma that innate-immune responses are nonspecific.[233]

The first member of Toll-like receptors (TLRs) – TLR 4 was described in 1997, which is a human homologue of the Drosophila Toll protein.[234] Nowadays at least 10 members of TLRs have been identified and they are grouped by their ligands (Table2). Toll-like receptors (TLRs) have been found as a key component of the innate immune system that detect microbial infection and trigger antimicrobial host defense responses. The basic function of TLRs is to help eliminate the invading pathogens and coordinate systemic defenses by activating multiple steps in the inflammatory reactions.[235] TLRs can specifically recognize various components from microbes including bacteria, virus and fungi. The binding of ligands to TLRs result in the activation of nuclear factor (NF)-kappaB followed by the synthesis of pro-inflammatory cytokines and chemokines and expression of adhesion molecules, which leads to acute inflammatory cellular infiltration consists of monocytes, neutrophils, basophils, eosinophils and NK cells.[236] However, overwhelming reaction and pro-inflammatory cytokine secretion can be detrimental to the host as well, which contributes to the pathogenesis of different diseases especially infections.[237,238] The expression of TLRs varies among different

individuals and cell types. It can be frequently found on epithelial cells lining the luminal tracts like intestinal epithelial cells.[239] Generally, the cell surface expression of TLRs is rather low, but can be upregulated by various stimuli.[240]

Table 2: Toll-like receptors and their major ligands

Receptor	Ligands	References
TLR 1	Tri-acyl lipopeptides (bacteria, mycobacteria)	235
TLR 2	Lipoprotein/lipopeptides (a variety of pathogens) Peptidoglycan (Gram-positive bacteria) Lipoteichoic acid (Gram-positive bacteria) Lipoarabinomannan (mycobacteria) Zymosan (fungi) CMV virions (virus)	235,241,242
TLR 3	Double-stranded RNA (virus)	235,241
TLR 4	LPS (Gram-negative bacteria) Lipoteichoic acid (Gram-positive bacteria) Fusion protein (virus) Evelope proteins (virus)	235,240,241
TLR 5	Flagellin (bacteria)	235,241
TLR 6	Lipopeptides (mycoplasma)	235,241
TLR 7	Imidazoquinoline (synthetic compounds)	235
TLR 8	Single-stranded RNA (virus)	242
TLR 9	CpG DNA (bacteria and virus)	235,241,242
TLR 10	Unknown	242

Not much work has been done about the role of TLRs in liver transplantation. It has been reported that TLR2 mRNA level is increased after ischemia-reperfusion injury in rat liver transplantation model indicating that TLR signaling pathway may be involved in post-transplant infections.[243] Activation of TLRs on Kupffer cells is one of the features during ischemia-reperfusion injury in liver transplantation.[244]

Thus, the exact mechanisms of liver allograft rejections are not yet elucidated. Although accumulated data indicates that cellular immunity plays a role in liver allograft rejections, there is a great paucity of data regarding the role of antibodies in mediating liver allograft rejections. Furthermore, biliary epithelial and liver endothelial cells have been suggested to play a more active role in immunological responses than was previously thought. To study the role played by human biliary epithelial cells and endothelial cells in immune-mediated responses within the liver, especially during allograft rejection, it is important to determine if these cells have the potential to interact with antibodies and T lymphocytes.

All these reasons led to the work described in this thesis. We were therefore interested in elucidating some of the mechanisms underlying liver allograft rejections. Specifically, we were interested in:

i) Detection and clinical correlation of the presence of antibodies in liver transplant patients to surface antigens expressed on the clinically relevant target cells of destruction and

ii) Determining the possible functional role of these antibodies in mediating liver allograft rejections.

iii) In addition, we also attempted to define the role of liver sinusoidal endothelial cells in allograft rejections in an experimental model.

2 Aims of the present study

The general aim of the study was to better understand the mechanisms involved in liver allograft rejections.

The specific aims were:

1) To determine the clinical importance of preformed antibodies to biliary epithelial cell in liver transplant recipients;

2) To study the clinical correlation of antibodies to biliary epithelial cell in the post-liver transplant period and their functional capacity;

3) To detect the presence of antibodies to sinusoidal endothelial cell in liver transplant recipients and investigate their clinical correlations and functional capacity;

4) To study the mechanisms underlying spontaneous rat liver allograft acceptance with a special emphasis on the role of liver sinusoidal endothelial cells.

3 Methodological considerations

The methods used in this thesis are described in the individual papers. Some methodological considerations are discussed below.

3.1 Patient population

Papers I and III were based on a patient population composed of 95 consecutive liver transplant patients performed at our center during 1999-2000. The indications for LTx, the surgical techniques and immunosuppression used in our center are comparable with others,[245,246]. In paper II we included 56 patients from whom blood samples were available both before and after transplantation, so this is a selected patient group from the original 95 patients.

3.2 Detection of antibodies

The detection of presence of antibodies in patient sera directed against donor or third party target cells can be performed using various methods. Two of the most common methods are the complement dependent cytotoxicity (CDC) assay or the more sensitive flow cytometric cross-match (FCXM) assay. Complement dependent cytotoxicity (CDC) assay is performed by incubating target cells with recipient serum, followed by complement addition and determination of cell killing.[247] This method is a functional test suitable for detection of high titered complement fixing antibodies. With the development of modern technologies, more sensitive crossmatching methods like flow cytometric crossmatch has been developed. In FCXM, target cells are first incubated with patient serum, followed by addition of fluorescein-labeled secondary anti-human antibodies. The target cell and antibody complexes can be detected by the flow cytometer. The advantage of FCXM is that it detects low titered complement fixing and non-complement fixing antibodies, but a 5-10% of false positive reaction has been reported.[105] This method is however not a functional test. Unfortunately neither of the two assays can distinguish between HLA and non-HLA antibodies and therefore assays for specificity determinations need to be performed to distinguish between these antibodies.

3.3 Determination of specificities of antibodies

HLA specificity: Many studies have confirmed that HLA antibodies are directly related with hyperacute rejections and also associated with acute rejection episodes and poor graft outcome in many organ transplants, so it is important to determine the HLA specificity of antibodies.[7,113-118,122,123]

Our group was the first to develop a novel specific method for HLA antibody detection with microbeads coated with pooled HLA class I or II antigens to specifically absorb away HLA antibodies from patient sera. Absorbed sera were then rechecked for binding to target cells, or alternatively, binding of antibodies to HLA-coated magnetic beads was detected with secondary antibodies.[248] Currently, HLA antigen-based ELISA (PRA-STAT), and HLA antigen-coated flow cytometry assay kit (FLOW-PRA) are

commercially available for detection of HLA specificity of antibodies present in patients sera.[6]

Based on specificity determinations, we found that most of the antibodies detected in our studies are non-HLA antibodies. However, it is important to mention that the antibodies detected in our studies are directed against third party target cells and not donor-specific cells. This limits the interpretation of the clinical significance of the antibodies detected in our studies.

Blood group antigen specificity: Presence of blood group antibodies are correlated with hyperacute rejections and poor graft survival.[130,131,133] We determined the presence of antibodies directed to blood group antigens by absorbing patient sera giving positive reactions with packed pooled red blood cells obtained from different healthy donors. The sera were then retested for binding to target cells and loss of reactivity indicated the presence of blood group antibodies. In our own experience, we have rarely detected the presence of these antibodies using cultured epithelial or endothelial cells.

Tissue specificity: In our studies we were interested in the detection of antibodies directed against liver specific cells such as liver epithelial and endothelial cells. To test whether the antibodies are tissue specific, we used human lung (bronchial) epithelial cells (LEC) and renal proximal tubular epithelial cells (RPTEC) as control target cells. Use of these control target cells help in determining the extent of cross-reactivity and relevance of the antibodies detected in sera of patients awaiting organ transplants.

3.4   Immunoglobulin classes

Only certain immunoglobulin classes are capable of activating complement, which include some IgG subclasses (IgG1 and IgG3 in humans) and IgM.[4] Most of the studies indicate that IgG antibodies are more important than IgM in organ transplantation.[249-251] The flow cytometric method for the detection of antibodies used in our study permits the detection of all immunoglobulin classes by applying different secondary antibodies.[252] In our studies, most of the sera from patients with rejections contained mixtures of both IgG and IgM antibodies, thus we are unable to confirm the finding that IgG antibodies are more deleterious than IgM.

3.5   Use of IgG F(ab')$_2$ fraction

Purified IgG fractions can be used for the detection or study of functional analysis of antibodies. The limitation of using whole IgG fractions is that the immunoglobulin molecule may bind the cells via the Fc fragment, thus excluding the study of true antigen-antibody binding mediated interactions and effects. Therefore, IgG F(ab')$_2$ fragments that lack Fc fraction are preferred for functional studies of antibodies.

3.6   Isolation of liver cells

Isolation of biliary epithelial cells was performed after a simple enzyme digestion using collagenase.[253] We found that the type of collagenase used and length of time for digestion was important in obtaining a good yield of these cells. In our experience use of Collagenase type IV and a time of 45-60 minutes were optimal for a good yield of

BEC. The BEC were isolated using magnetic particles coated with an antibody (HEA-125) directed to a pan epithelial cell marker expressed on BEC but not on hepatocytes. This approach for isolation of BEC is relatively simple, easy and not time consuming. These cells can be maintained phenotypically and morphologically stable for up to seven-eight passages.

Isolation of liver sinusoidal endothelial cells on the other hand is more tedious and time-consuming.[230] In our experience, the yield of LSEC was also found to be affected by the nature of the enzyme used for tissue degradation. Use of a mild enzyme such as dispase and incubation at 4°C overnight gave a good yield of LSEC. Enzyme digestion was followed by mechanical detachment of LSEC from the pieces of chopped liver and separation on a percoll gradient. Although the method of separation is tedious, a 100% success rate of LSEC isolation was observed on every occasion. LSEC can be passaged for 5-6 times with stable phenotype and morphology as determined by flow cytometry and electron microscopy.

These two liver cell populations provide a unique *in vitro* system to study the biology of BEC and LSEC in normal conditions as well as inflammatory processes in various liver disorders.

3.7   Rat liver transplantation model

In paper IV we used a rat liver transplantation model to study the role of LSEC function in liver allograft rejection and tolerance. Rat liver transplantation model is a well established and widely accepted model for preservation as well as immunological study of liver transplantation.[254] Unlike humans, rat liver allografts can survive without reconstruction of arterial flow. However, more and more studies showed that arterial reconstruction is important.[255,256] In our study, arterialized orthotopic liver transplantation was performed under inhalation anesthesia with isoflurane according to the method described by Liu et al.[257] Briefly, livers taken from donors were preserved in 4°C Ringer's solution before grafting into the recipient. In the recipient, the anastomosis of suprahepatic vena cava was performed by 7-0 running suture. The portal vein and infrahepatic vena cava was reconstructed by the cuff technique and bile duct was connected by end-to-end anastomosis over an indwelling stent (Fig. 4). Rearterialization was achieved between donor common hepatic artery and recipient proper artery using "sleeve" technique under microscope.[257]

In this model liver transplants from DA to LEW rats are rejected, but liver grafts from LEW to DA were spontaneously accepted. This is a good model which permits the study of mechanisms of acute rejections and tolerance by comparing the rejection and acceptance animals. However, it should be kept in mind that in humans, spontaneous acceptance of liver allograft normally does not occur. Animal experimental models are important tools that enable study of important biological phenomena that may be extrapolated to the human system. However, due to physiological differences, sometimes the results obtained from animal studies may not be directly applicable in humans.
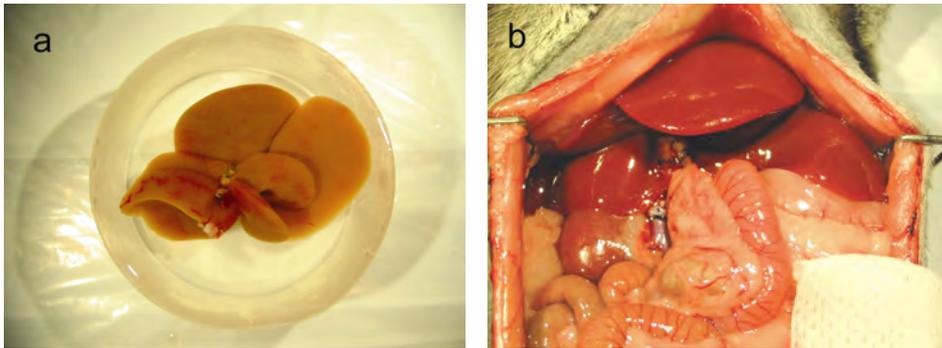
Figure 4. Rat liver transplantation model. a, Liver graft is in preservation before transplantation. b, Liver is implanted into the recipient.

4 Results and Discussion

The results from the four papers are briefly presented, followed by some general discussions.

4.1 Antibodies to BEC and LSEC in LTx

In paper I and II we found that preformed antibodies to BEC are associated with acute liver allograft rejections and antibodies to BEC in the post-transplant period are associated with cholangitis. We further demonstrated that post-transplant antibodies to BEC are capable of inducing TLR 2 and 3 expressions on BEC and production of proinflammatory cytokines and chemokines, which could contribute to the pathogenesis of cholangitis. Antibody depositions were found in liver biopsies from liver transplanted patients during rejection and cholangitis episodes. In paper III we found that antibodies to LSEC are significantly correlated to acute liver allograft rejections and antibodies to LSEC can induce costimulatory molecule expression and down-regulate TGF-β production by LSEC. As a result, T cell proliferation is greatly increased, which constitutes a novel mechanism of acute liver allograft rejection. Majority of antibodies to BEC and LSEC are not HLA antibodies and they do not cross react with other control cell types. To our knowledge, these are the first series of studies that describe the clinical importance of tissue-specific antibodies in liver transplanted patients. Our results show that non-HLA antibodies reactive with BEC or LSEC are closely associated with acute liver allograft rejections and our *in vitro* experiments revealed novel mechanisms by which antibodies may contribute to acute rejection episodes, suggesting that maybe it is time to reevaluate the significance of humoral immunity in acute liver allograft rejections. In addition, our results also confirmed the observation that donor-specific lymphocyte crossmatch per se without specificity determinations did not correlate with acute liver allograft rejections.[113-115]

The specificity of the antibodies to BEC and LSEC, namely which cell surface antigens these antibodies recognize, remains to be defined. These antibodies are not directed against blood group antigens and they do not cross react with control cell types. Further experiments using immunoprecipitation will provide us more detailed information about the specificities of these antibodies.

The presence of pre-transplant alloantibodies is usually caused by repeated blood transfusion, loss of a previous transplant or multiple pregnancies.[258-260] Majority of patients included in our studies do not have a history of alloimmunization, so we assume that the antibodies to BEC and LSEC detected in our studies are autoantibodies. In some of the patients these antibodies could not be detected after transplantation. There might be several reasons for this. First of all, the liver can eliminate antibodies via endocytosis, which makes the antibody titer too low to be detected in the serum.[127] Furthermore, antibody titer fluctuation over time may explain the lack of antibody detection.[4] The large volume of blood replacement during liver transplant surgery will dilute circulating antibodies and reduce the number of host B lymphocytes.[97]

Post-transplant antibodies could be heterogeneous in terms of their specificity. They could be a mixture of antibodies detected already in the pre-transplant period as well as newly developed alloantibodies after allo-liver implantation. It has been reported that de novo HLA antibodies in the post-transplant period is associated with increased acute and chronic rejection and decreased graft survival in several kinds of organ transplants.[125] It could be argued that the presence of antibodies in the post-transplant period is just a phenomenon secondary to the key cellular events that are the real factors responsible for the rejection. However, the fact that these antibodies can be detected before rejection episodes and graft failure, favors the hypothesis that these antibodies may initiate a rejection process.[122,125] The complicated nature of post-transplant antibodies and the lack of knowledge about their specificity may contribute to the difference in clinical associations observed with pre- and post-transplant antibodies to BEC.

In our studies we also found that some patients had antibodies to BEC or LSEC but did not develop acute rejections. A number of factors may play a role. Antibody titer, affinity and avidity are important factors influencing antibody-antigen interaction and their functional ability. It can be speculated that the titer, affinity and avidity of antibodies to BEC or LSEC may be different in patients with rejections as compared to those without rejections. In addition, the impact of immunosuppressive drugs routinely used in liver transplant patients on these antibody-involving rejections is not clear. We also found patients who did not have antibodies to BEC or LSEC developed acute rejection episodes. Acute rejection response is a complicated process, which involves both cellular and humoral immunity via many mechanisms.[63] Antibodies to BEC and LSEC in acute rejection process is just one actor in the concert.

4.2   How do antibodies to BEC and LSEC contribute to acute liver allograft rejections

Apart from the classical effector functions antibodies may (Fig. 5):

Facilitate antigen presenting capacity of LSEC by induction of an important co-stimulatory molecule: Our in vitro experiments showed that antibodies to LSEC may participate in acute rejection process indirectly by regulating the function of effector T cells, in this particular case by inducing costimulatory molecule CD86 expression on LSEC. It has been proposed that LSEC may function as non-professional antigen presenting cells, but human LSEC express only MHC class I & II and CD40, without constitutive expression of costimulatory molecules CD80 or CD86.[30] To our knowledge, this is the first report which demonstrates that antibodies may induce expression of CD86 on a cell type.

Possess immunomodulatory function: TGF-β is a cytokine constitutively produced by LSEC and is well known for suppressing T cell proliferation.[261] We demonstrate that binding of antibodies to LSEC down-regulates TGF-β level, thereby enhancing the cellular immune responses. Therefore, increased T cell proliferation during liver allograft rejections could be a result of combined antigen presentation by LSEC with decreased TGF-β levels. Previous results from our group showed that antibodies to
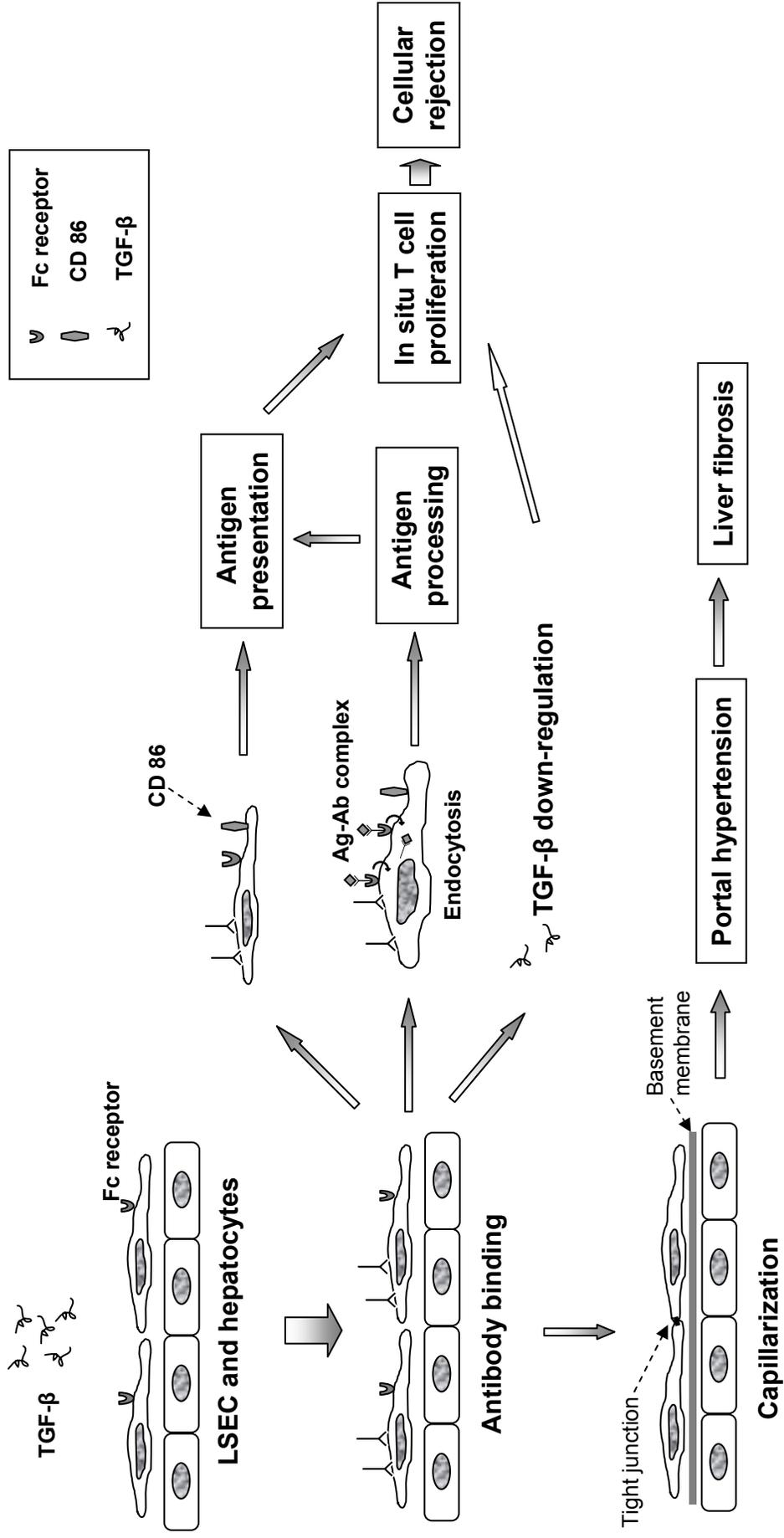
Figure 5. Facilitation of liver allograft rejection by antibodies to LSEC

BEC are capable of inducing adhesion molecule CD44 expression and proinflammatory cytokine IL-6 production, indicating the antibodies to BEC may indirectly contribute to inflammation process by its immunomodulatory function.[216] Furthermore, binding of antibodies may activate endothelial and epithelial cells as demonstrated by upregulation of activating markers such as HLA DR expression, adhesion molecules such as VCAM-1, ICAM-1 and E-selectin, all of which may help facilitate cellular immune responses.

Facilitate antigen-uptake by LSEC: LSEC are considered to be one of the most efficient scavengers of the human body. The scavenger function of LSEC is mainly mediated by four groups of endocytic receptors, namely mannose receptor, hyaluronan/scavenger receptor, collagen-α chain receptor and Fc-γ receptor.[224] Thus, we speculate that antibodies binding to an antigen expressed on the LSEC of the transplanted liver allograft may facilitate uptake of the antigen and presentation to allospecific host T cells. In this way antibodies may facilitate direct antigen presentation locally in the liver.

Change morphology and phenotype of LSEC: Our group has previously shown that antibodies binding to LSEC can change the morphology and phenotype of liver sinusoidal endothelial cell to a vascular one.[230] The antibodies are able to capillarize the sinusoidal endothelial cells by inducing production of basement membrane, loss of fenestrae and formation of tight junctions. They further induced expression of markers such as Factor VIII RAg and CD31 that LSEC normally do not express. The unique arrangement of the normal sinusoidal endothelium is likely to facilitate the large exchanges that take place between hepatocytes and the blood. It is known that the formation of basement membrane and changes in LSEC will interfere with the bi-directional exchange of molecules and therefore have deleterious effects on liver physiology, such as decreased sinusoidal compliance with increased resistance to blood flow and may contribute to development of portal hypertension.[262] Other consequences that may result from these changes may be the development of cirrhosis by causing ischemic atrophy of hepatocytes, thereby leading to increased fibrogenesis and compensatory hypertrophy of surrounding hepatocytes.[263] All these changes may result in the development of hepatocellular failure. Thus, morphological transformation of LSEC to vascular-type endothelial cells in patients with LSEC antibodies may have important clinical consequences. Capillarization of LSEC in liver allografts have been reported during rejections and graft failure, thus antibodies to LSEC may mediate liver allograft rejections by this mechanism.[264]

4.3 Clinical implications of BEC and LSEC antibody detection

Our studies demonstrate that patients with antibodies to BEC or LSEC have increased risks for developing acute rejections after LTx. This emphasizes the need to use BEC and LSEC as target cells for crossmatching in liver transplantation. By using a panel of BEC and LSEC isolated from different donors we will be able to assess the risk for acute rejections as soon as the patient is accepted on the waiting list. Although a positive antibody reaction may not necessarily prevent transplantation, strategies in tailoring immunosuppression may be considered. In addition, marginal livers, which

are vulnerable to ischemia-reperfusion injury and may have a compromised functional capacity, may not be suitable donor livers for patients with antibodies to BEC or LSEC.

Our routine immunosuppression protocol with different drug combinations in liver transplantation is mainly targeted for cellular immune responses. Our findings that antibodies may participate in acute liver allograft rejections prompt us to consider in some patients, especially BEC or LSEC antibody positive patients, immunosuppression combinations which inhibit humoral immunity like tacrolimus together with mycophenolate mofetil should be applied.[191-193] B cell targeting therapy, like immunoadsorption with protein A, plasmapheresis, IVIg, or anti-CD20 monoclonal antibodies, should also be considered.

4.4 Experimental *in vivo* data implicating a role for LSEC in rejection of liver allografts

In paper IV, we used a liver transplantation model in Lewis (LEW) and DA rats. These two rat strains are fully MHC mismatched and have no syndromes of immunodeficiency. Interestingly, liver transplantations from DA to LEW rats were rejected as expected, but liver allografts from Lewis to DA rats were accepted without immunosuppression – transplant tolerance (verified by our unpublished results showing that liver implantation protects subsequent small bowel grafts which are otherwise rejected). Extensive investigations using these two combinations of rat liver transplants revealed a significant difference in serum hyaluronic acid (HA) level especially in the early post-transplant period, which implicates a role for the endocytic functional capacity of LSEC (Fig. 6). Ischemia and reperfusion injury is a common reason for LSEC dysfunction early after transplantation.[265] The cold ischemia time in all of our experiments was carefully controlled to be the same to ensure that LSEC in different group of animals were exposed to the same insult from ischemia-reperfusion injury. Yet, significantly different HA levels were observed. This implicates that LSEC in DA and LEW rats may be differentially susceptible to ischemia-reperfusion injury. However, the fact that HA level in LEW to DA tolerance group is even lower than DA to DA or LEW to LEW syngeneic transplant groups may indicate the involvement of factors other than ischemia-reperfusion injury. Investigation with DA and LEW rats showed a basic difference in serum cytokine IL-1β level, which is high in DA rats but not detectable in LEW rats. IL-1β is shown to promote LSEC endocytic function.[266] Therefore, the basic IL-1β difference in the two strains of animals may help to explain the significant difference of HA level in rejection and tolerance groups.

Another significant difference between rejection and tolerance group is serum IL-2 levels, which we speculate could be a reflection of development of regulatory T cells in the acceptance group. The question arises as to the role of LSEC in the establishment of tolerance in LEW to DA rat combination. It is well known that innate immunity has great impact on adaptive immune response by various mechanisms.[227] LSEC have strong clearance function via endocytosis removing circulating physiological waste as well as particles like IgG-immune complex, which constitutes a major part of innate immunity of the liver.[225] So the decrease of LSEC endocytic function in the early post-LTx period, an impairment of innate immunity of the liver, may be an important reason leading to graft rejection. On the other hand, it has been shown that antigen

presentation by mouse LSEC to T cells results in tolerance instead of rejection.[52] In our study, the impairment of endocytic function of LSEC we found by HA level may also be a reflection of their decreased general functional status including antigen presenting capacity. Thus, early post-transplant dysfunction of LSEC might be one factor leading to liver allograft rejection. Therefore, strategies to reduce ischemia-reperfusion injury and protocols that help maintain or improve LSEC functions should be further explored in liver transplantation.
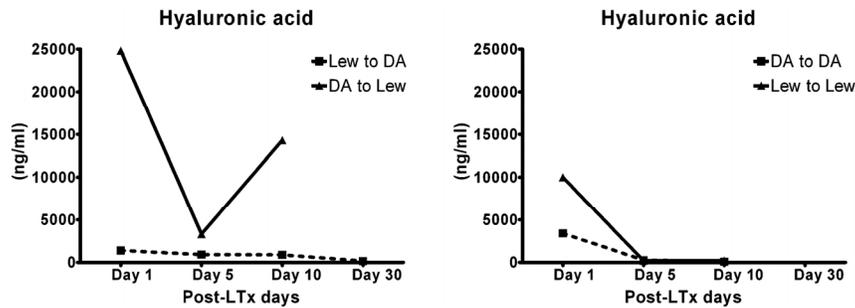

Figure 6. Hyaluronic acid level in different group of liver transplanted rats

5 Conclusions

- Preformed antibodies to BEC are associated with acute rejections in liver transplantation. The presence of these antibodies prior to transplantation may facilitate acute liver allograft rejections.

- Antibodies to BEC found in the post-LTx period are associated with cholangitis. *In vitro* experiments showed that antibodies to BEC are capable of inducing TLR 2 & 3 protein expression, and production of proinflammatory cytokines and chemokines. Thus, antibodies to BEC via those mechanisms may facilitate epithelial inflammatory response to microbial components and contribute to post-transplant cholangitis

- Antibodies to LSEC are significantly associated with acute rejections in liver transplantation. Antibodies to LSEC may facilitate acute liver rejections by inducing CD86 expression and down-regulating TGF-β level, thus, up-regulating T-cell proliferation.

- Preserved functional capacity of liver sinusoidal endothelial cell function may be important for spontaneous acceptance of liver allografts in rats. Strategies to reduce ischemia-reperfusion injury and preservation protocols that help maintain LSEC functional integrity may be beneficial for liver transplant outcome.

6 Future perspectives

Our work describes a novel role for tissue-specific antibodies in acute liver allograft rejections and also an important role for LSEC in liver transplantation. However, there are still many questions remaining to be answered:

- The specificity, namely which antigens are recognized by anti-BEC and LSEC antibodies, needs to be defined in future experiments using immunoprecipitation;

- The polymorphism and titer kinetics of antibodies to BEC and LSEC need to be clarified by well-planned studies;

- The relationship between C4d staining and antibodies to BEC and LSEC needs to be explored;

- Screening of patients before LTx with a panel of BEC and LSEC isolated from several different healthy donors to assess the risk of developing acute rejections may be considered in multicenter prospective clinical trials;

- The effect of B cell targeting therapy in BEC and LSEC antibody positive patients may need to be evaluated in future studies;

- In rat liver transplantation models, approaches that specifically destroy LSEC function and methods to improve LSEC function like giving exogenous IL-1β should be performed to confirm our findings;

- In human LTx, methods for better preservation of LSEC function should be further explored.

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8 References

1. Rinaldi E: The first homoplastic limb transplant according to the legend of Saint Cosmas and Saint Damian. Ital J Orthop Traumatol 1987, 13:393-406

2. Morris PJ: Transplantation--a medical miracle of the 20th century. N Engl J Med 2004, 351:2678-2680

3. Merrill JP, Murray JE, Harrison JH, Guild WR: Successful homotransplantation of the human kidney between identical twins. J Am Med Assoc 1956, 160:277-282

4. Goldsby RA, Kindt TJ, Osbourne BA, Kuby J: Kuby immunology. New York, W.H. Freeman, 2000, pp xxv, 670 s.

5. Michaels PJ, Fishbein MC, Colvin RB: Humoral rejection of human organ transplants. Springer Semin Immunopathol 2003, 25:119-140

6. Sumitran-Holgersson S: HLA-specific alloantibodies and renal graft outcome. Nephrol Dial Transplant 2001, 16:897-904

7. Terasaki PI: Humoral theory of transplantation. Am J Transplant 2003, 3:665-673

8. Neuberger J: Incidence, timing, and risk factors for acute and chronic rejection. Liver Transpl Surg 1999, 5:S30-36

9. Libby P, Pober JS: Chronic rejection. Immunity 2001, 14:387-397

10. Goodman J, Mohanakumar T: Chronic rejection: failure of immune regulation. Front Biosci 2003, 8:s838-844

11. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The U.S. Multicenter FK506 Liver Study Group. N Engl J Med 1994, 331:1110-1115.

12. Williams R, Neuhaus P, Bismuth H, McMaster P, Pichlmayr R, Calne R, Otto G, Groth C: Two-year data from the European multicentre tacrolimus (FK506) liver study. Transpl Int 1996, 9:S144-150.

13. Mayer AD, Dmitrewski J, Squifflet JP, Besse T, Grabensee B, Klein B, Eigler FW, Heemann U, Pichlmayr R, Behrend M, Vanrenterghem Y, Donck J, van Hooff J, Christiaans M, Morales JM, Andres A, Johnson RW, Short C, Buchholz B, Rehmert N, Land W, Schleibner S, Forsythe JL, Talbot D, Pohanka E, et al.: Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. Transplantation 1997, 64:436-443

14. Margreiter R: Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. Lancet 2002, 359:741-746

15. Lakkis FG: Transplantation tolerance: a journey from ignorance to memory. Nephrol Dial Transplant 2003, 18:1979-1982

16. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR: Homotransplantation of the Liver in Humans. Surg Gynecol Obstet 1963, 117:659-676

17. Southern PB, Davies MH: Indication and assessment for liver transplantation. Clin Med 2002, 2:313-316

18. Tra TT, Nissen N, Poordad FF, Martin P: Advances in liver transplantation. New strategies and current care expand access, enhance survival. Postgrad Med 2004, 115:73-76, 79-85

19.   Kamada N: The immunology of experimental liver transplantation in the rat. Immunology 1985, 55:369-389

20.   Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA: Induction of immunological tolerance by porcine liver allografts. Nature 1969, 223:472-476

21.   Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE: Murine liver allograft transplantation: tolerance and donor cell chimerism. Hepatology 1994, 19:916-924

22.   Zhong R, He G, Sakai Y, Li XC, Garcia B, Wall W, Duff J, Stiller C, Grant D: Combined small bowel and liver transplantation in the rat: possible role of the liver in preventing intestinal allograft rejection. Transplantation 1991, 52:550-552

23.   Garside P, Mowat AM: Mechanisms of oral tolerance. Crit Rev Immunol 1997, 17:119-137

24.   Gorczynski RM, Chan Z, Chung S, Cohen Z, Levy G, Sullivan B, Fu XM: Prolongation of rat small bowel or renal allograft survival by pretransplant transfusion and/or by varying the route of allograft venous drainage. Transplantation 1994, 58:816-820

25.   Opelz G, Wujciak T, Dohler B, Scherer S, Mytilineos J: HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet 1999, 1:334-342

26.   Calne RY: Immunological tolerance--the liver effect. Immunol Rev 2000, 174:280-282

27.   Chiba S, Goto S, Shimizu Y, Vari F, Lord R, Edwards-Smith C, Kobayashi S, Ochiai T, Isono K: The characterization of reconstituted passenger leukocytes on the induction of tolerance in rat liver transplantation. Transpl Int 1997, 10:350-356

28.   Asakura H, Ku G, Kataoka M, Flye MW: Regulatory cells develop after the spontaneous acceptance of rat liver allografts. Surgery 2004, 136:532-536

29.   Knolle PA, Gerken G: Local control of the immune response in the liver. Immunol Rev 2000, 174:21-34

30.   Knolle PA, Limmer A: Neighborhood politics: the immunoregulatory function of organ-resident liver endothelial cells. Trends Immunol 2001, 22:432-437

31.   Bird G, Friend P, Donaldson P, O'Grady J, Portmann B, Calne R, Williams R: Hyperacute rejection in liver transplantation: a case report. Transplant Proc 1989, 21:3742-3744.

32.   Imagawa DK, Noguchi K, Iwaki Y, Busuttil RW: Hyperacute rejection following ABO-compatible orthotopic liver transplantation--a case report. Transplantation 1992, 54:1114-1117.

33.   Legmann P, Dousset B, Tudoret L, Hazebroucq V, Girardot C, Lemonnier EF, Houssin D, Bonnin A: Hyperacute rejection in liver transplantation: CT findings. J Comput Assist Tomogr 1994, 18:139-142

34.   Hathaway M, Gunson BK, Keogh AC, Briggs D, McMaster P, Neuberger JM: A positive crossmatch in liver transplantation--no effect or inappropriate analysis? A prospective study. Transplantation 1997, 64:54-59

35.   Molajoni ER, Cinti P, Orlandini A, Molajoni J, Tugulea S, Ho E, Liu Z, Suciu-Foca N, Cortesini R: Mechanism of liver allograft rejection: the indirect recognition pathway. Hum Immunol 1997, 53:57-63

36.   Sawada T, Shimizu A, Kubota K, Fuchinoue S, Teraoka S: Lobular damage caused by cellular and humoral immunity in liver allograft rejection. Clin Transplant 2005, 19:110-114

37. Krukemeyer MG, Moeller J, Morawietz L, Rudolph B, Neumann U, Theruvath T, Neuhaus P, Krenn V: Description of B lymphocytes and plasma cells, complement, and chemokines/receptors in acute liver allograft rejection. Transplantation 2004, 78:65-70

38. Neil DA, Hubscher SG: Histologic and biochemical changes during the evolution of chronic rejection of liver allografts. Hepatology 2002, 35:639-651

39. Middleton D: Current and emerging technology for HLA typing. Int J Hematol 2002, 76 Suppl 2:150-151

40. Morris PJ, Johnson RJ, Fuggle SV, Belger MA, Briggs JD: Analysis of factors that affect outcome of primary cadaveric renal transplantation in the UK. HLA Task Force of the Kidney Advisory Group of the United Kingdom Transplant Support Service Authority (UKTSSA). Lancet 1999, 354:1147-1152

41. Petersdorf EW, Mickelson EM, Anasetti C, Martin PJ, Woolfrey AE, Hansen JA: Effect of HLA mismatches on the outcome of hematopoietic transplants. Curr Opin Immunol 1999, 11:521-526

42. Poli F, Scalamogna M, Aniasi A, Brambilla C, Cardillo M, Crespiatico L, Diomelli B, Pedranzini L, Sirchia G: A retrospective evaluation of HLA-A, B and -DRB1 matching in liver transplantation. Transpl Int 1998, 11 Suppl 1:S347-349

43. Hankey KG, Drachenberg CB, Papadimitriou JC, Klassen DK, Philosophe B, Bartlett ST, Groh V, Spies T, Mann DL: MIC expression in renal and pancreatic allografts. Transplantation 2002, 73:304-306

44. Jiang S, Herrera O, Lechler RI: New spectrum of allorecognition pathways: implications for graft rejection and transplantation tolerance. Curr Opin Immunol 2004, 16:550-557

45. Hollander GA, Zuklys S, Forster E, Krenger W: On costimulatory signals and T cell tolerance: relevance for transplantation immunity. Transplant Proc 1999, 31:25S-31S

46. Grewal IS, Flavell RA: CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 1998, 16:111-135

47. Jaiswal AI, Dubey C, Swain SL, Croft M: Regulation of CD40 ligand expression on naive CD4 T cells: a role for TCR but not co-stimulatory signals. Int Immunol 1996, 8:275-285

48. Lenschow DJ, Walunas TL, Bluestone JA: CD28/B7 system of T cell costimulation. Annu Rev Immunol 1996, 14:233-258

49. Forbes RD, Parfrey NA, Gomersall M, Darden AG, Guttmann RD: Dendritic cell-lymphoid cell aggregation and major histocompatibility antigen expression during rat cardiac allograft rejection. J Exp Med 1986, 164:1239-1258

50. Demetris AJ, Qian S, Sun H, Fung JJ, Yagihashi A, Murase N, Iwaki Y, Gambrell B, Starzl TE: Early events in liver allograft rejection. Delineation of sites of simultaneous intragraft and recipient lymphoid tissue sensitization. Am J Pathol 1991, 138:609-618

51. Knolle PA, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW: Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology 1999, 116:1428-1440

52. Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA: Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med 2000, 6:1348-1354

53. Parker DC, Greiner DL, Phillips NE, Appel MC, Steele AW, Durie FH, Noelle RJ, Mordes JP, Rossini AA: Survival of mouse pancreatic islet allografts in

recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. Proc Natl Acad Sci U S A 1995, 92:9560-9564

54. Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, Cho HR, Aruffo A, Hollenbaugh D, Linsley PS, Winn KJ, Pearson TC: Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996, 381:434-438

55. Sun H, Subbotin V, Chen C, Aitouche A, Valdivia LA, Sayegh MH, Linsley PS, Fung JJ, Starzl TE, Rao AS: Prevention of chronic rejection in mouse aortic allografts by combined treatment with CTLA4-Ig and anti-CD40 ligand monoclonal antibody. Transplantation 1997, 64:1838-1843

56. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Jr., Knechtle SJ: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci U S A 1997, 94:8789-8794

57. Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Buchanan K, Fechner JH, Jr., Germond RL, Kampen RL, Patterson NB, Swanson SJ, Tadaki DK, TenHoor CN, White L, Knechtle SJ, Harlan DM: Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat Med 1999, 5:686-693

58. Elster EA, Hale DA, Mannon RB, Cendales LC, Swanson SJ, Kirk AD: The road to tolerance: renal transplant tolerance induction in nonhuman primate studies and clinical trials. Transpl Immunol 2004, 13:87-99

59. Bartlett AS, McCall JL, Ameratunga R, Howden B, Ramadas R, Yeong ML, Benjamin CD, Hess D, Munn SR: Intragraft gene and protein expression in rat liver allografts treated with costimulatory blockade alone or in combination with CyA. J Surg Res 2003, 115:1-8

60. Bartlett AS, McCall JL, Ameratunga R, Howden B, Yeong ML, Benjamin CD, Hess D, Peach R, Munn SR: Costimulatory blockade prevents early rejection, promotes lymphocyte apoptosis, and inhibits the upregulation of intragraft interleukin-6 in an orthotopic liver transplant model in the rat. Liver Transpl 2002, 8:458-468

61. Olthoff KM, Gelman AE, Zamir GA, Debonera F, Krasinkas AM, Shen X, Gao F, Shaked A: Pattern of alloimmune response in second same donor allografts after induction of tolerance using CTLA4Ig. Transplantation 2001, 72:1952-1956

62. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, Qian S: Costimulation blockade promotes the apoptotic death of graft-infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. Transplantation 2001, 72:1423-1432

63. Rocha PN, Plumb TJ, Crowley SD, Coffman TM: Effector mechanisms in transplant rejection. Immunol Rev 2003, 196:51-64

64. Arakelov A, Lakkis FG: The alloimmune response and effector mechanisms of allograft rejection. Semin Nephrol 2000, 20:95-102

65. Adams D: Mechanisms of liver allograft rejection in man. Clin Sci (Lond) 1990, 78:343-350

66. Egawa H, Martinez OM, Quinn MB, Villanueva JC, So S, Esquivel CO, Krams SM: Acute liver allograft rejection in the rat. An analysis of the immune response. Transplantation 1995, 59:97-102

67. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997, 25:658-663

68.   Krams SM, Villanueva JC, Quinn MB, Martinez OM: Expression of the cytotoxic T cell mediator granzyme B during liver allograft rejection. Transpl Immunol 1995, 3:162-166

69.   Gershon RK: A disquisition on suppressor T cells. Transplant Rev 1975, 26:170-185

70.   Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995, 155:1151-1164

71.   Taams L, Vukmanovic-Stejic M, Salmon M, Akbar A: Immune regulation by CD4+CD25+ regulatory T cells: implications for transplantation tolerance. Transpl Immunol 2003, 11:277-285

72.   Hori S, Nomura T, Sakaguchi S: Control of regulatory T cell development by the transcription factor Foxp3. Science 2003, 299:1057-1061

73.   Papiernik M, de Moraes ML, Pontoux C, Vasseur F, Penit C: Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. Int Immunol 1998, 10:371-378

74.   Hall BM, Pearce NW, Gurley KE, Dorsch SE: Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. III. Further characterization of the CD4+ suppressor cell and its mechanisms of action. J Exp Med 1990, 171:141-157

75.   Taylor PA, Noelle RJ, Blazar BR: CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J Exp Med 2001, 193:1311-1318

76.   Bolton EM: Regulatory T cells in transplantation: natural or induced? Transplantation 2005, 79:643-645

77.   Benghiat FS, Graca L, Braun MY, Detienne S, Moore F, Buonocore S, Flamand V, Waldmann H, Goldman M, Le Moine A: Critical influence of natural regulatory CD25+ T cells on the fate of allografts in the absence of immunosuppression. Transplantation 2005, 79:648-654

78.   Thornton AM, Shevach EM: Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 2000, 164:183-190

79.   Lee MKt, Moore DJ, Markmann JF: Regulatory CD4+CD25+T cells in prevention of allograft rejection. Front Biosci 2003, 8:s968-981

80.   Koenen HJ, Joosten I: Blockade of CD86 and CD40 induces alloantigen-specific immunoregulatory T cells that remain anergic even after reversal of hyporesponsiveness. Blood 2000, 95:3153-3161

81.   Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L: Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. J Immunol 2001, 167:1945-1953

82.   Bluestone JA: Opinion: Regulatory T-cell therapy: is it ready for the clinic? Nat Rev Immunol 2005, 5:343-349

83.   Kataoka M, Margenthaler JA, Ku G, Eilers M, Flye MW: "Infectious tolerance" develops after the spontaneous acceptance of Lewis-to-Dark Agouti rat liver transplants. Surgery 2003, 134:227-234

84.   Yoshizawa A, Ito A, Li Y, Koshiba T, Sakaguchi S, Wood KJ, Tanaka K: The roles of CD25+CD4+ regulatory T cells in operational tolerance after living donor liver transplantation. Transplant Proc 2005, 37:37-39

85.   Tanabe K: Calcineurin inhibitors in renal transplantation: what is the best option? Drugs 2003, 63:1535-1548

86.   Morelon E, Mamzer-Bruneel MF, Peraldi MN, Kreis H: Sirolimus: a new promising immunosuppressive drug. Towards a rationale for its use in renal transplantation. Nephrol Dial Transplant 2001, 16:18-20

87.   Chan GL, Canafax DM, Johnson CA: The therapeutic use of azathioprine in renal transplantation. Pharmacotherapy 1987, 7:165-177

88.   Becker BN: Mycophenolate mofetil. Transplant Proc 1999, 31:2777-2778

89.   Perry I, Neuberger J: Immunosuppression: towards a logical approach in liver transplantation. Clin Exp Immunol 2005, 139:2-10

90.   Waldmann TA, O'Shea J: The use of antibodies against the IL-2 receptor in transplantation. Curr Opin Immunol 1998, 10:507-512

91.   Norman DJ, Leone MR: The role of OKT3 in clinical transplantation. Pediatr Nephrol 1991, 5:130-136

92.   Grillo-Lopez AJ: Rituximab: an insider's historical perspective. Semin Oncol 2000, 27:9-16

93.   Neuberger J: Developments in liver transplantation. Gut 2004, 53:759-768

94.   Demirkiran A, Kok A, Kwekkeboom J, Metselaar HJ, Tilanus HW, van der Laan LJ: Decrease of CD4(+)CD25(+) T Cells in Peripheral Blood After Liver Transplantation: Association With Immunosuppression. Transplant Proc 2005, 37:1194-1196

95.   Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Riethmuller G, Land W, Albert E: Capillary deposition of C4d complement fragment and early renal graft loss. Kidney Int 1993, 43:1333-1338

96.   Racusen LC: Antibody-mediated rejection in the kidney. Transplant Proc 2004, 36:768-769

97.   Donaldson PT, Williams R: Cross-matching in liver transplantation. Transplantation 1997, 63:789-794.

98.   Janeway CA: Immunobiology : the immune system in health and disease. New York, NY, Garland Pub : Churchill Livingstone, 2001, pp xviii, 732 s.

99.   Karuppan SS, Lindholm A, Moller E: Fewer acute rejection episodes and improved outcome in kidney- transplanted patients with selection criteria based on crossmatching. Transplantation 1992, 53:666-673.

100.   Turco MC, de Felice M, Corbo L, Giarrusso PC, Yang SY, Ferrone S, Venuta S: Enhancing effect of anti-HLA class I monoclonal antibodies on T cell proliferation induced via CD2 molecule. J Immunol 1988, 141:2275-2281

101.   Geppert TD, Wacholtz MC, Patel SS, Lightfoot E, Lipsky PE: Activation of human T cell clones and Jurkat cells by cross-linking class I MHC molecules. J Immunol 1989, 142:3763-3772

102.   Turco MC, De Felice M, Corbo L, Morrone G, Mertelsmann R, Ferrone S, Venuta S: Regulatory role of a monomorphic determinant of HLA Class I antigens in T cell proliferation. J Immunol 1985, 135:2268-2273

103.   Terasaki PI, McClelland JD: Microdroplet Assay of Human Serum Cytotoxins. Nature 1964, 204:998-1000

104.   Mittal KK, Mickey MR, Singal DP, Terasaki PI: Serotyping for homotransplantation. 18. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation 1968, 6:913-927

105.   Iwaki Y, Lau M, Cook DJ, Takemoto S, Terasaki PI: Crossmatching with B and T cells and flow cytometry. Clin Transpl 1986:277-284

106.   Creemers P, Brink J, Kahn D: Interaction between panel reactive antibodies, auto- and cold reactive antibodies, and a positive B cell cross-match in renal and cardiac allograft survival. Clin Transplant 1997, 11:134-138

107. Karuppan SS, Lindholm A, Moller E: Characterization and significance of donor-reactive B cell antibodies in current sera of kidney transplant patients. Transplantation 1990, 49:510-515

108. Mahoney RJ, Taranto S, Edwards E: B-Cell crossmatching and kidney allograft outcome in 9031 United States transplant recipients. Hum Immunol 2002, 63:324-335

109. Lobashevsky AL, Senkbeil RW, Shoaf J, Mink C, Rowe C, Lobashevsky ES, Burke R, Hudson S, Deierhoi MH, Thomas JM: Specificity of preformed alloantibodies causing B cell positive flow crossmatch in renal transplantation. Clin Transplant 2000, 14:533-542

110. Le Bas-Bernardet S, Hourmant M, Valentin N, Paitier C, Giral-Classe M, Curry S, Follea G, Soulillou JP, Bignon JD: Identification of the antibodies involved in B-cell crossmatch positivity in renal transplantation. Transplantation 2003, 75:477-482

111. Buscaroli A, Nanni Costa A, Iannelli S, Cianciolo G, De Santis L, La Manna G, Stefoni S, Vangelista A, Bonomini V: Value of panel reactive antibodies (PRA) as a guide to the treatment of hyperimmunized patients in renal transplantation. Transpl Int 1992, 5 Suppl 1:S54-57

112. Takaya S, Bronsther O, Iwaki Y, Nakamura K, Abu-Elmagd K, Yagihashi A, Demetris AJ, Kobayashi M, Todo S, Tzakis AG, et al.: The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors. Transplantation 1992, 53:400-406.

113. Lobo PI, Spencer C, Douglas MT, Stevenson WC, Pruett TL: The lack of long-term detrimental effects on liver allografts caused by donor-specific anti-HLA antibodies. Transplantation 1993, 55:1063-1066.

114. Nikaein A, Backman L, Jennings L, Levy MF, Goldstein R, Gonwa T, Stone MJ, Klintmalm G: HLA compatibility and liver transplant outcome. Improved patient survival by HLA and cross-matching. Transplantation 1994, 58:786-792.

115. Donaldson PT, Thomson LJ, Heads A, Underhill JA, Vaughan RW, Rolando N, Williams R: IgG donor-specific crossmatches are not associated with graft rejection or poor graft survival after liver transplantation. An assessment by cytotoxicity and flow cytometry. Transplantation 1995, 60:1016-1023.

116. Manez R, Kelly RH, Kobayashi M, Takaya S, Bronsther O, Kramer D, Duquesnoy RJ, Iwaki Y, Fung JJ, Starzl TE, et al.: Immunoglobulin G lymphocytotoxic antibodies in clinical liver transplantation: studies toward further defining their significance. Hepatology 1995, 21:1345-1352

117. Kasahara M, Kiuchi T, Takakura K, Uryuhara K, Egawa H, Asonuma K, Uemoto S, Inomata Y, Ohwada S, Morishita Y, Tanaka K: Postoperative flow cytometry crossmatch in living donor liver transplantation: clinical significance of humoral immunity in acute rejection. Transplantation 1999, 67:568-575

118. Takakura K, Kiuchi T, Kasahara M, Inomata Y, Tanaka K: Humoral immunity in acute cellular rejection after living-donor liver transplantation. Transplant Proc 1999, 31:526-527

119. Iwatsuki S, Rabin BS, Shaw BW, Jr., Starzl TE: Liver transplantation against T cell-positive warm crossmatches. Transplant Proc 1984, 16:1427-1429.

120. Gordon RD, Fung JJ, Markus B, Fox I, Iwatsuki S, Esquivel CO, Tzakis A, Todo S, Starzl TE: The antibody crossmatch in liver transplantation. Surgery 1986, 100:705-715

121. Batts KP, Moore SB, Perkins JD, Wiesner RH, Grambsch PM, Krom RA: Influence of positive lymphocyte crossmatch and HLA mismatching on vanishing bile duct syndrome in human liver allografts. Transplantation 1988, 45:376-379

122. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW: Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. Transplantation 2003, 75:1034-1040

123. Vasilescu ER, Ho EK, de la Torre L, Itescu S, Marboe C, Cortesini R, Suciu-Foca N, Mancini D: Anti-HLA antibodies in heart transplantation. Transpl Immunol 2004, 12:177-183

124. Terasaki PI, Kreisler M, Mickey RM: Presensitization and kidney transplant failures. Postgrad Med J 1971, 47:89-100

125. McKenna RM, Takemoto SK, Terasaki PI: Anti-HLA antibodies after solid organ transplantation. Transplantation 2000, 69:319-326

126. Terasaki PI, Ozawa M: Predicting kidney graft failure by HLA antibodies: a prospective trial. Am J Transplant 2004, 4:438-443

127. Rifle G, Mousson C, Martin L, Guignier F, Hajji K: Donor-specific antibodies in allograft rejection: clinical and experimental data. Transplantation 2005, 79:S14-18

128. Sumitran-Holgersson S, Wilczek HE, Holgersson J, Soderstrom K: Identification of the nonclassical HLA molecules, mica, as targets for humoral immunity associated with irreversible rejection of kidney allografts. Transplantation 2002, 74:268-277

129. Klintmalm GB, Nery JR, Husberg BS, Gonwa TA, Tillery GW: Rejection in liver transplantation. Hepatology 1989, 10:978-985.

130. Ratner LE, Phelan D, Brunt EM, Mohanakumar T, Hanto DW: Probable antibody-mediated failure of two sequential ABO-compatible hepatic allografts in a single recipient. Transplantation 1993, 55:814-819

131. Rego J, Prevost F, Rumeau JL, Modesto A, Fourtanier G, Durand D, Suc JM, Ohayon E, Ducos J: Hyperacute rejection after ABO-incompatible orthotopic liver transplantation. Transplant Proc 1987, 19:4589-4590.

132. Starzl TE, Tzakis A, Makowka L, Banner B, Demetrius A, Ramsey G, Duquesnoy R, Griffin M: The definition of ABO factors in transplantation: relation to other humoral antibody states. Transplant Proc 1987, 19:4492-4497

133. Gugenheim J, Samuel D, Reynes M, Bismuth H: Liver transplantation across ABO blood group barriers. Lancet 1990, 336:519-523

134. Stegall MD, Dean PG, Gloor JM: ABO-incompatible kidney transplantation. Transplantation 2004, 78:635-640

135. Tyden G, Kumlien G, Fehrman I: Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. Transplantation 2003, 76:730-731

136. Morioka D, Sekido H, Kubota K, Sugita M, Tanaka K, Togo S, Yamanaka S, Sasaki T, Inayama Y, Shimada H: Antibody-mediated rejection after adult ABO-incompatible liver transplantation remedied by gamma-globulin bolus infusion combined with plasmapheresis. Transplantation 2004, 78:1225-1228

137. Usuda M, Fujimori K, Koyamada N, Fukumori T, Sekiguchi S, Kawagishi N, Akamatsu Y, Enomoto Y, Satoh K, Satoh A, Ishida K, Moriya T, Satomi S: Successful use of anti-CD20 monoclonal antibody (rituximab) for ABO-incompatible living-related liver transplantation. Transplantation 2005, 79:12-16

138. Nakamura Y, Matsuno N, Iwamoto H, Yokoyama T, Kuzuoka K, Kihara Y, Taira S, Sagara T, Jojima Y, Konno O, Tashiro J, Akashi I, Hama K, Narumi K, Iwahori T, Uchiyama M, Tanaka K, Nagao T: Successful case of adult ABO-incompatible liver transplantation: beneficial effects of intrahepatic artery infusion therapy: a case report. Transplant Proc 2004, 36:2269-2273

139. Ahern AT, Artruc SB, DellaPelle P, Cosimi AB, Russell PS, Colvin RB, Fuller TC: Hyperacute rejection of HLA-AB-identical renal allografts associated with B lymphocyte and endothelial reactive antibodies. Transplantation 1982, 33:103-106

140. Pober JS, Orosz CG, Rose ML, Savage CO: Can graft endothelial cells initiate a host anti-graft immune response? Transplantation 1996, 61:343-349

141. Brasile L, Rodman E, Shield CF, 3rd, Clarke J, Cerilli J: The association of antivascular endothelial cell antibody with hyperacute rejection: a case report. Surgery 1986, 99:637-640

142. Sumitran-Karuppan S, Tyden G, Reinholt F, Berg U, Moller E: Hyperacute rejections of two consecutive renal allografts and early loss of the third transplant caused by non-HLA antibodies specific for endothelial cells. Transpl Immunol 1997, 5:321-327

143. Perrey C, Brenchley PE, Johnson RW, Martin S: An association between antibodies specific for endothelial cells and renal transplant failure. Transpl Immunol 1998, 6:101-106

144. Faulk WP, Rose M, Meroni PL, Del Papa N, Torry RJ, Labarrere CA, Busing K, Crisp SJ, Dunn MJ, Nelson DR: Antibodies to endothelial cells identify myocardial damage and predict development of coronary artery disease in patients with transplanted hearts. Hum Immunol 1999, 60:826-832

145. Crisp SJ, Dunn MJ, Rose ML, Barbir M, Yacoub MH: Antiendothelial antibodies after heart transplantation: the accelerating factor in transplant-associated coronary artery disease? J Heart Lung Transplant 1994, 13:81-91; discussion 91-82

146. Brasile L, Zerbe T, Rabin B, Clarke J, Abrams A, Cerilli J: Identification of the antibody to vascular endothelial cells in patients undergoing cardiac transplantation. Transplantation 1985, 40:672-675.

147. Mauiyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Tolkoff-Rubin NE, Williams WW, Delmonico FL, Cosimi AB, Colvin RB: Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. J Am Soc Nephrol 2002, 13:779-787

148. Vermehren D, Sumitran-Holgersson S: Isolation of precursor endothelial cells from peripheral blood for donor-specific crossmatching before organ transplantation. Transplantation 2002, 74:1479-1486

149. Sumitran-Holgersson S, Ge X, Karrar A, Xu B, Nava S, Broome U, Nowak G, Ericzon BG: A novel mechanism of liver allograft rejection facilitated by antibodies to liver sinusoidal endothelial cells. Hepatology 2004, 40:1211-1221

150. Cerilli GJ, Brasile L, Galouzis T, DeFrancis ME: Clinical significance of antimonocyte antibody in kidney transplant recipients. Transplantation 1981, 32:495-497

151. Smith JD, Crisp SJ, Dunn MJ, Pomerance A, Yacoub MH, Rose ML: Pre-transplant anti-epithelial cell antibodies and graft failure after single lung transplantation. Transpl Immunol 1995, 3:68-73.

152. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schonemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G: Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005, 352:558-569

153. Jurcevic S, Ainsworth ME, Pomerance A, Smith JD, Robinson DR, Dunn MJ, Yacoub MH, Rose ML: Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. Transplantation 2001, 71:886-892

154. Dubel L, Farges O, Johanet C, Sebagh M, Bismuth H: High incidence of antitissue antibodies in patients experiencing chronic liver allograft rejection. Transplantation 1998, 65:1072-1075

155. Duclos-Vallee JC, Johanet C, Bach JF, Yamamoto AM: Autoantibodies associated with acute rejection after liver transplantation for type-2 autoimmune hepatitis. J Hepatol 2000, 33:163-166

156. Gehrs BC, Friedberg RC: Autoimmune hemolytic anemia. Am J Hematol 2002, 69:258-271

157. Bartunkova J, Tesar V, Sediva A: Diagnostic and pathogenetic role of antineutrophil cytoplasmic autoantibodies. Clin Immunol 2003, 106:73-82

158. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 2002, 110:955-963

159. McFarlane IG: Autoimmune liver diseases. Scand J Clin Lab Invest Suppl 2001, 235:53-60

160. Zetterman RK: Autoimmunity and alcoholic liver disease. Am J Med 1990, 89:127-128.

161. Inui A, Sogo T, Komatsu H, Miyakawa H, Fujisawa T: Antibodies against cytokeratin 8/18 in a patient with de novo autoimmune hepatitis after living-donor liver transplantation. Liver Transpl 2005, 11:504-507

162. Neuberger J: Recurrent primary biliary cirrhosis. Liver Transpl 2003, 9:539-546

163. Jin YP, Jindra PT, Gong KW, Lepin EJ, Reed EF: Anti-HLA class I antibodies activate endothelial cells and promote chronic rejection. Transplantation 2005, 79:S19-21

164. Magil AB, Tinckam K: Monocytes and peritubular capillary C4d deposition in acute renal allograft rejection. Kidney Int 2003, 63:1888-1893

165. Yard B, Spruyt-Gerritse M, Claas F, Thorogood J, Bruijn JA, Paape ME, Stein SY, van Es LA, van Bockel JH, Kooymans-Coutinho M, et al.: The clinical significance of allospecific antibodies against endothelial cells detected with an antibody-dependent cellular cytotoxicity assay for vascular rejection and graft loss after renal transplantation. Transplantation 1993, 55:1287-1293

166. Moeller J, Krukemeyer MG, Morawietz L, Schmeding M, Dankof A, Neumann U, Krenn V, Berek C: Molecular case report: IgVH analysis in acute humoral and cellular liver allograft rejection suggests a selected accumulation of effector B cells and plasma cells. Virchows Arch 2005, 446:325-332

167. Lee PC, Terasaki PI, Takemoto SK, Lee PH, Hung CJ, Chen YL, Tsai A, Lei HY: All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. Transplantation 2002, 74:1192-1194

168. McKane BW, Trulock EP, Patterson GA, Mohanakumar T: Lung transplantation and bronchiolitis obliterans: an evolution in understanding. Immunol Res 2001, 24:177-190

169. Fujita T, Endo Y, Nonaka M: Primitive complement system--recognition and activation. Mol Immunol 2004, 41:103-111

170. Feucht HE: Complement C4d in graft capillaries -- the missing link in the recognition of humoral alloreactivity. Am J Transplant 2003, 3:646-652

171. Feucht HE, Felber E, Gokel MJ, Hillebrand G, Nattermann U, Brockmeyer C, Held E, Riethmuller G, Land W, Albert E: Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. Clin Exp Immunol 1991, 86:464-470

172. Crespo M, Pascual M, Tolkoff-Rubin N, Mauiyyedi S, Collins AB, Fitzpatrick D, Farrell ML, Williams WW, Delmonico FL, Cosimi AB, Colvin RB, Saidman SL: Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics. Transplantation 2001, 71:652-658

173. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, Cecka MJ, Cosyns JP, Demetris AJ, Fishbein MC, Fogo A, Furness P, Gibson IW, Glotz D, Hayry P, Hunsickern L, Kashgarian M, Kerman R, Magil AJ, Montgomery R, Morozumi K, Nickeleit V, Randhawa P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 2003, 3:708-714

174. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB: Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 2001, 12:574-582

175. Theruvath TP, Saidman SL, Mauiyyedi S, Delmonico FL, Williams WW, Tolkoff-Rubin N, Collins AB, Colvin RB, Cosimi AB, Pascual M: Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. Transplantation 2001, 72:77-83

176. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, Watschinger B, Kerjaschki D, Exner M: Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. J Am Soc Nephrol 2002, 13:2371-2380

177. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ: Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. J Am Soc Nephrol 2002, 13:242-251

178. Rodriguez ER: The pathology of heart transplant biopsy specimens: revisiting the 1990 ISHLT working formulation. J Heart Lung Transplant 2003, 22:3-15

179. Behr TM, Feucht HE, Richter K, Reiter C, Spes CH, Pongratz D, Uberfuhr P, Meiser B, Theisen K, Angermann CE: Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies. J Heart Lung Transplant 1999, 18:904-912

180. Enomoto K, Nishikawa Y, Omori Y, Tokairin T, Yoshida M, Ohi N, Nishimura T, Yamamoto Y, Li Q: Cell biology and pathology of liver sinusoidal endothelial cells. Med Electron Microsc 2004, 37:208-215

181. Bellamy MC, Gedney JA, Buglass H, Gooi JH: Complement membrane attack complex and hemodynamic changes during human orthotopic liver transplantation. Liver Transpl 2004, 10:273-278

182. Herzenberg AM, Gill JS, Djurdjev O, Magil AB: C4d deposition in acute rejection: an independent long-term prognostic factor. J Am Soc Nephrol 2002, 13:234-241

183. Glotz D, Antoine C, Julia P, Pegaz-Fiornet B, Duboust A, Boudjeltia S, Fraoui R, Combes M, Bariety J: Intravenous immunoglobulins and transplantation for patients with anti-HLA antibodies. Transpl Int 2004, 17:1-8

184. Snanoudj R, Beaudreuil S, Arzouk N, de Preneuf H, Durrbach A, Charpentier B: Immunological strategies targeting B cells in organ grafting. Transplantation 2005, 79:S33-36

185. Kriaa F, Laurian Y, Hiesse C, Tchernia G, Charpentier B: Five years' experience at one centre with protein A immunoadsorption in patients with deleterious allo/autoantibodies (anti-HLA antibodies, autoimmune bleeding

disorders) and post-transplant patients relapsing with focal glomerular sclerosis. Nephrol Dial Transplant 1995, 10 Suppl 6:108-110

186. Bohmig GA, Regele H, Exner M, Derhartunian V, Kletzmayr J, Saemann MD, Horl WH, Druml W, Watschinger B: C4d-positive acute humoral renal allograft rejection: effective treatment by immunoadsorption. J Am Soc Nephrol 2001, 12:2482-2489

187. White NB, Greenstein SM, Cantafio AW, Schechner R, Glicklich D, McDonough P, Pullman J, Mohandas K, Boctor F, Uehlinger J, Tellis V: Successful rescue therapy with plasmapheresis and intravenous immunoglobulin for acute humoral renal transplant rejection. Transplantation 2004, 78:772-774

188. Gloor JM, DeGoey SR, Pineda AA, Moore SB, Prieto M, Nyberg SL, Larson TS, Griffin MD, Textor SC, Velosa JA, Schwab TR, Fix LA, Stegall MD: Overcoming a positive crossmatch in living-donor kidney transplantation. Am J Transplant 2003, 3:1017-1023

189. Becker YT, Becker BN, Pirsch JD, Sollinger HW: Rituximab as treatment for refractory kidney transplant rejection. Am J Transplant 2004, 4:996-1001

190. Vieira CA, Agarwal A, Book BK, Sidner RA, Bearden CM, Gebel HM, Roggero AL, Fineberg NS, Taber T, Kraus MA, Pescovitz MD: Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1. Safety, pharmacodynamics, and pharmacokinetics. Transplantation 2004, 77:542-548

191. Kimball JA, Pescovitz MD, Book BK, Norman DJ: Reduced human IgG anti-ATGAM antibody formation in renal transplant recipients receiving mycophenolate mofetil. Transplantation 1995, 60:1379-1383

192. Smith KG, Isbel NM, Catton MG, Leydon JA, Becker GJ, Walker RG: Suppression of the humoral immune response by mycophenolate mofetil. Nephrol Dial Transplant 1998, 13:160-164

193. Pascual M, Saidman S, Tolkoff-Rubin N, Williams WW, Mauiyyedi S, Duan JM, Farrell ML, Colvin RB, Cosimi AB, Delmonico FL: Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. Transplantation 1998, 66:1460-1464

194. Sonnenday CJ, Warren DS, Cooper M, Samaniego M, Haas M, King KE, Shirey RS, Simpkins CE, Montgomery RA: Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. Am J Transplant 2004, 4:1315-1322

195. Vierling JM: Immunologic mechanisms of hepatic allograft rejection. Semin Liver Dis 1992, 12:16-27

196. Knolle PA, Limmer A: Control of immune responses by savenger liver endothelial cells. Swiss Med Wkly 2003, 133:501-506

197. Mohanakumar T, Rhodes C, Mendez-Picon G, Flye MW, Lee HM: Antiidiotypic antibodies to human major histocompatibility complex class I and II antibodies in hepatic transplantation and their role in allograft survival. Transplantation 1987, 44:54-58

198. Baldwin WM, 3rd, Sanfilippo F: Antibodies and graft rejection. Transplant Proc 1989, 21:605-608

199. Demetris AJ, Murase N, Nakamura K, Iwaki Y, Yagihashi A, Valdivia L, Todo S, Iwatsuki S, Takaya S, Fung JJ, et al.: Immunopathology of antibodies as effectors of orthotopic liver allograft rejection. Semin Liver Dis 1992, 12:51-59

200. Platt JL: C4d and the fate of organ allografts. J Am Soc Nephrol 2002, 13:2417-2419

201. Wang N, Lee JM, Tobiasch E, Csizmadia E, Smith NR, Gollackes B, Robson SC, Bach FH, Lin Y: Induction of xenograft accommodation by modulation of elicited antibody responses1 2. Transplantation 2002, 74:334-345

202. Dalmasso AP, Benson BA, Johnson JS, Lancto C, Abrahamsen MS: Resistance against the membrane attack complex of complement induced in porcine endothelial cells with a Gal alpha(1-3)Gal binding lectin: up-regulation of CD59 expression. J Immunol 2000, 164:3764-3773

203. Thethy S, Thomson B, Pleass H, Wigmore SJ, Madhavan K, Akyol M, Forsythe JL, James Garden O: Management of biliary tract complications after orthotopic liver transplantation. Clin Transplant 2004, 18:647-653

204. Li S, Stratta RJ, Langnas AN, Wood RP, Marujo W, Shaw BW, Jr.: Diffuse biliary tract injury after orthotopic liver transplantation. Am J Surg 1992, 164:536-540

205. Lillemoe KD, Pitt HA, Cameron JL: Current management of benign bile duct strictures. Adv Surg 1992, 25:119-174

206. Kibbler CC: Infections in liver transplantation: risk factors and strategies for prevention. J Hosp Infect 1995, 30 Suppl:209-217

207. Grant AG, Billing BH: The isolation and characterization of a bile ductule cell population from normal and bile-duct ligated rat livers. Br J Exp Pathol 1977, 58:301-310

208. Wu CT, Davis PA, Luketic VA, Gershwin ME: A review of the physiological and immunological functions of biliary epithelial cells: targets for primary biliary cirrhosis, primary sclerosing cholangitis and drug-induced ductopenias. Clin Dev Immunol 2004, 11:205-213

209. Vierling JM: Immune disorders of the liver and bile duct. Gastroenterol Clin North Am 1992, 21:427-449

210. Steinhoff G, Wonigeit K, Pichlmayr R: Analysis of sequential changes in major histocompatibility complex expression in human liver grafts after transplantation. Transplantation 1988, 45:394-401

211. Leon MP, Bassendine MF, Gibbs P, Thick M, Kirby JA: Immunogenicity of biliary epithelium: study of the adhesive interaction with lymphocytes. Gastroenterology 1997, 112:968-977

212. Scholz M, Gooss A, Blaheta RA, Encke A, Markus BH: Cultured human biliary epithelial cells induce allogeneic lymphocyte activation in vitro: possible relevance in liver transplant rejection. Immunol Lett 1997, 55:27-34

213. Sundstrom JB, Ansari AA: Comparative study of the role of professional versus semiprofessional or nonprofessional antigen presenting cells in the rejection of vascularized organ allografts. Transpl Immunol 1995, 3:273-289

214. Adams DH, Hubscher SG, Shaw J, Rothlein R, Neuberger JM: Intercellular adhesion molecule 1 on liver allografts during rejection. Lancet 1989, 2:1122-1125

215. Fung JJ, Zeevi A, Starzl TE, Demetris J, Iwatsuki S, Duquesnoy RJ: Functional characterization of infiltrating T lymphocytes in human hepatic allografts. Hum Immunol 1986, 16:182-199

216. Xu B, Broome U, Ericzon BG, Sumitran-Holgersson S: High frequency of autoantibodies in patients with primary sclerosing cholangitis that bind biliary epithelial cells and induce expression of CD44 and production of interleukin 6. Gut 2002, 51:120-127

217. Yasuda M, Nakano K, Yasumoto K, Tanaka Y: CD44: functional relevance to inflammation and malignancy. Histol Histopathol 2002, 17:945-950

218. Meyer zum Buschenfelde KH, Lohse AW, Manns M, Poralla T: Autoimmunity and liver disease. Hepatology 1990, 12:354-363

219. Cruickshank SM, Southgate J, Selby PJ, Trejdosiewicz LK: Expression and cytokine regulation of immune recognition elements by normal human biliary epithelial and established liver cell lines in vitro. J Hepatol 1998, 29:550-558

220. Blomhoff R, Holte K, Naess L, Berg T: Newly administered [3H]retinol is transferred from hepatocytes to stellate cells in liver for storage. Exp Cell Res 1984, 150:186-193

221. MacPhee PJ, Schmidt EE, Groom AC: Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy. Am J Physiol 1995, 269:G692-698

222. Wisse E, Braet F, Luo D, De Zanger R, Jans D, Crabbe E, Vermoesen A: Structure and function of sinusoidal lining cells in the liver. Toxicol Pathol 1996, 24:100-111

223. Schlepper-Schafer J, Hulsmann D, Djovkar A, Meyer HE, Herbertz L, Kolb H, Kolb-Bachofen V: Endocytosis via galactose receptors in vivo. Ligand size directs uptake by hepatocytes and/or liver macrophages. Exp Cell Res 1986, 165:494-506

224. Svistounov D, Smedsrod B: Hepatic clearance of advanced glycation end products (AGEs)--myth or truth? J Hepatol 2004, 41:1038-1040

225. Smedsrod B: Clearance function of scavenger endothelial cells. Comp Hepatol 2004, 3 Suppl 1:S22

226. Fox A, Harrison LC: Innate immunity and graft rejection. Immunol Rev 2000, 173:141-147

227. Medzhitov R, Janeway CA, Jr.: Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 1997, 9:4-9

228. Crispe IN, Dao T, Klugewitz K, Mehal WZ, Metz DP: The liver as a site of T-cell apoptosis: graveyard, or killing field? Immunol Rev 2000, 174:47-62

229. Park S, Murray D, John B, Crispe IN: Biology and significance of T-cell apoptosis in the liver. Immunol Cell Biol 2002, 80:74-83

230. Xu B, Broome U, Uzunel M, Nava S, Ge X, Kumagai-Braesch M, Hultenby K, Christensson B, Ericzon BG, Holgersson J, Sumitran-Holgersson S: Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. Am J Pathol 2003, 163:1275-1289

231. Hoebe K, Janssen E, Beutler B: The interface between innate and adaptive immunity. Nat Immunol 2004, 5:971-974

232. Medzhitov R, Janeway C, Jr.: Innate immunity. N Engl J Med 2000, 343:338-344

233. Netea MG, van der Graaf C, Van der Meer JW, Kullberg BJ: Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. J Leukoc Biol 2004, 75:749-755

234. Means TK, Golenbock DT, Fenton MJ: The biology of Toll-like receptors. Cytokine Growth Factor Rev 2000, 11:219-232

235. Takeda K, Kaisho T, Akira S: Toll-like receptors. Annu Rev Immunol 2003, 21:335-376

236. Iwasaki A, Medzhitov R: Toll-like receptor control of the adaptive immune responses. Nat Immunol 2004, 5:987-995

237. Cook DN, Pisetsky DS, Schwartz DA: Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004, 5:975-979

238.   Read RC, Wyllie DH: Toll receptors and sepsis. Curr Opin Crit Care 2001, 7:371-375

239.   Cario E, Podolsky DK: Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000, 68:7010-7017

240.   Janssens S, Beyaert R: Role of Toll-like receptors in pathogen recognition. Clin Microbiol Rev 2003, 16:637-646

241.   Lien E, Ingalls RR: Toll-like receptors. Crit Care Med 2002, 30:S1-S11

242.   Abreu MT, Arditi M: Innate immunity and toll-like receptors: clinical implications of basic science research. J Pediatr 2004, 144:421-429

243.   Tsoulfas G, Takahashi Y, Ganster RW, Yagnik G, Guo Z, Fung JJ, Murase N, Geller DA: Activation of the lipopolysaccharide signaling pathway in hepatic transplantation preservation injury. Transplantation 2002, 74:7-13

244.   Fondevila C, Busuttil RW, Kupiec-Weglinski JW: Hepatic ischemia/reperfusion injury--a fresh look. Exp Mol Pathol 2003, 74:86-93

245.   Wiesner R, Rabkin J, Klintmalm G, McDiarmid S, Langnas A, Punch J, McMaster P, Kalayoglu M, Levy G, Freeman R, Bismuth H, Neuhaus P, Mamelok R, Wang W: A randomized double-blind comparative study of mycophenolate mofetil and azathioprine in combination with cyclosporine and corticosteroids in primary liver transplant recipients. Liver Transpl 2001, 7:442-450

246.   Jain A, Kashyap R, Dodson F, Kramer D, Hamad I, Khan A, Eghestad B, Starzl TE, Fung JJ: A prospective randomized trial of tacrolimus and prednisone versus tacrolimus, prednisone and mycophenolate mofetil in primary adult liver transplantation: a single center report. Transplantation 2001, 72:1091-1097

247.   Karuppan S LA, Moller E: Pre- and post-transplant sensitization in cyclosporin A-treated patients. Clin Transplantation 1988, 2:245-251

248.   Sumitran-Karuppan S, Moller E: The use of magnetic beads coated with soluble HLA class I or class II proteins in antibody screening and for specificity determinations of donor-reactive antibodies. Transplantation 1996, 61:1539-1545.

249.   Chapman JR, Taylor CJ, Ting A, Morris PJ: Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches. Relationship to renal transplant outcome. Transplantation 1986, 42:608-613

250.   Roelen DL, van Bree J, Witvliet MD, Habraken I, van Beelen E, van't Sant PH, de Waal LP, van Rood JJ, Claas FH: IgG antibodies against an HLA antigen are associated with activated cytotoxic T cells against this antigen, IgM are not. Transplantation 1994, 57:1388-1392

251.   Taylor CJ, Chapman JR, Ting A, Morris PJ: Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome. Transplantation 1989, 48:953-958

252.   Karuppan SS, Ohlman S, Moller E: The occurrence of cytotoxic and non-complement-fixing antibodies in the crossmatch serum of patients with early acute rejection episodes. Transplantation 1992, 54:839-844

253.   Joplin R, Strain AJ, Neuberger JM: Immuno-isolation and culture of biliary epithelial cells from normal human liver. In Vitro Cell Dev Biol 1989, 25:1189-1192.

254.   Kashfi A, Mehrabi A, Pahlavan PS, Schemmer P, Gutt CN, Friess H, Gebhard MM, Schmidt J, Buchler MW, Kraus TW: A review of various techniques of orthotopic liver transplantation in the rat. Transplant Proc 2005, 37:185-188

255. Post S, Menger MD, Rentsch M, Gonzalez AP, Herfarth C, Messmer K: The impact of arterialization on hepatic microcirculation and leukocyte accumulation after liver transplantation in the rat. Transplantation 1992, 54:789-794

256. Zhao D, Wheatley AM: Orthotopic liver transplantation in the rat: comparison of models with and without rearterialization of the graft. Eur Surg Res 1993, 25:294-302

257. Liu T, Freise CE, Ferrell L, Ascher NL, Roberts JP: A modified vascular "sleeve" anastomosis for rearterialization in orthotopic liver transplantation in rats. Transplantation 1992, 54:179-180

258. Dausset J: Leuco-agglutinins and blood transfusion. Leuco-agglutinins, Vox Scag, 1954, pp 190

259. Payne R RM: Fetomaternal leukocyte incompatibility. J Clin Invest 1958, 37:1756

260. Walford RL, Carter PK, Anderson RE: Leukocyte antibodies following skin homografting in the human. Transplant Bull 1962, 29:16-18

261. Moustakas A, Pardali K, Gaal A, Heldin CH: Mechanisms of TGF-beta signaling in regulation of cell growth and differentiation. Immunol Lett 2002, 82:85-91

262. Oda M, Han JY, Nakamura M: Endothelial cell dysfunction in microvasculature: relevance to disease processes. Clin Hemorheol Microcirc 2000, 23:199-211

263. Le Couteur DG, Fraser R, Hilmer S, Rivory LP, McLean AJ: The hepatic sinusoid in aging and cirrhosis: effects on hepatic substrate disposition and drug clearance. Clin Pharmacokinet 2005, 44:187-200

264. Noguchi K, Yagihashi A, Kobayashi M, Yoshida Y, Tanaka K, Konno A, Hayashi S, Terasawa K, Nakamura K, Demetris AJ, et al.: Capillarization of the hepatic sinusoid in failed liver grafts. Transplant Proc 1993, 25:1110

265. Huet PM, Nagaoka MR, Desbiens G, Tarrab E, Brault A, Bralet MP, Bilodeau M: Sinusoidal endothelial cell and hepatocyte death following cold ischemia-warm reperfusion of the rat liver. Hepatology 2004, 39:1110-1119

266. Asumendi A, Alvarez A, Martinez I, Smedsrod B, Vidal-Vanaclocha F: Hepatic sinusoidal endothelium heterogeneity with respect to mannose receptor activity is interleukin-1 dependent. Hepatology 1996, 23:1521-1529