Division of Hepatology and Transplantation

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"The Division is dedicated to translational research: bringing the laboratory to the bedside."

INTRODUCTION

The Division is strategically based on the Denmark Hill campus to take advantage of the excellent opportunities at King's College Hospital for transferring research findings in the laboratory directly to the bedside. The Hospital serves a large local population and is also a tertiary referral centre for liver disease and liver transplantation and an internationally recognised centre for diabetes.

The Paediatric Liver Service is the largest paediatric liver unit in the world with some 3000 admissions per year, including more than 300 new patients. The Adult Liver Service admits more than 3000 new patients each year. The combined Adult and Paediatric liver transplantation programme is the largest in Europe, having performed some 2300 transplants since 1990, and many of the new surgical techniques have been pioneered at King's – including reduced liver, split-liver and living-related transplantation. The most recent developments have been in cell transplantation, using isolated human liver cells (hepatocytes) to treat inherited metabolic liver disorders and pancreatic islet cells for treating diabetes (see IN FOCUS section).

The Division's research programme focuses on the development of new techniques for diagnosis, understanding mechanisms of tissue damage and improving treatment – including genetic screening, gene therapy, anti-cancer vaccines, and targeted immunosuppression to prevent rejection in transplant recipients and to control autoimmune diseases. Disturbances in metabolism and nutrition are a common consequence of disease and seriously impact on potential for recovery. A major objective is the improvement of understanding of the mechanisms of such metabolic disturbances (including central control of metabolism, lung function, and insulin resistance).

REPORT

Research strategy

This is strongly focused on translational research to combine clinical observation with basic science for the benefit of patients with complex liver and metabolic disorders. The common interests of the hepatology, liver transplantation, diabetes, clinical sciences and renal groupings have created a natural cooperation between the groups, which has led to collaborative research programmes resulting in highly innovative therapeutic approaches. The Division's research objectives span six main areas: cell transplantation (Amiel, Dhawan, Heaton, Huang, Hughes, Mieli-Vergani), liver immunopathology and endocrine autoimmunity (Banga, Christie, Fabre, McFarlane, Mieli-Vergani, Vergani, Wendon), cancer immunotherapy (Collins, Fabre, Harrison), molecular genetics (Bomford, Hendry, Thompson, Tredger), gene therapy (Collins, Fabre, Hendry), and physiology and metabolism (Amiel, Miell, Wendon).

A major development being considered is a translational research unit for cellular therapy which will include hepatocyte, islet

STAFF LIST

CS Clinical Sciences; **D&E** Diabetes, Endocrinology and Internal Medicine; **LST** Liver Studies & Transplantation; **N** Nephrology and Transplantation; **#** Also PhD/MD/MS candidates

PROFESSORS

Professor Stephanie Amiel (Clinical) **D&E**; Professor John Fabre (Clinical) **CS**; Professor Bruce Hendry (Clinical) **N**; Professor Bernard Portmann (Clinical) **LST**; Professor Diego Vergani (Clinical) **LST**

READERS

Dr Paul Banga **D&E**; Dr Adrian Bomford (Clinical) **LST**; Dr Michael Christie **D&E**

SENIOR LECTURERS

Dr Anil Dhawan (Clinical, Honorary) **LST**; Dr Nedim Hadzic (Clinical, Honorary) **LST**; Dr Phillip Harrison (Clinical) **LST**; Mr Nigel Heaton (Clinical, Honorary) **LST**; Dr Robin Hughes **LST**; Ian McFarlane (Honorary) **LST**; Dr John Miell (Clinical) **D&E**; Dr Dr John O'Grady (Clinical, Honorary) **LST**; Mr Mohamed Rela (Clinical, Honorary) **LST**; Dr Richard Thompson (Clinical) **LST**; Dr J.Michael Tredger **LST**; Dr Julia Wendon (Clinical) **LST**

LECTURERS

Dr Emma Bingham (Clinical) **D&E #**; Dr Louise Collins **CS**; Dr Guo Cai Huang **D&E**; Dr Claire Sharpe (Clinical, Honorary) **N**

RESEARCH FELLOWS

Dr Cristiana Almerighi **LST**; Dr Karen Anthony **D&E #**; Dr Charalambos Antoniades (Clinical) **LST**; Dr Antony Antoniou (Clinical) **LST**; Dr Adrian Barnardo (Clinical) **LST**; Dr Jane Byrne **LST #**; Dr Helena Daniels **LST**; Dr Sheetal Dhadphale **LST**; Dr Xue-Bin Dong **CS**; Dr Matthew Foxton (Clinical) **LST**; Dr Ina Geissler **CS**; Dr Jacqueline Gilbert (Clinical) **D&E**; Dr Raffaele Girlanda (Clinical) **LST**; Dr Munther Hussain **LST**; Dr Wayel Jassem (Clinical) **LST #**; Dr Peter Langley **LST**; Dr Kristian Lobner **D&E #**; Dr Yun Ma **LST**; Dr Rifat Malik **D&E #**; Dr Ragai Mitry **LST**; Dr Jonathan Mitchell (Clinical) **LST #**; Dr Tina Muehlburg (Clinical) **D&E**; Dr Philip Murphy **LST**; Dr James O'Beirne (Clinical) **LST #**; Dr Prakash Rao (Clinical) **D&E #**; Dr Paul Richardson (Clinical) **CS #**; Dr Graham Roberts **D&E #**; Dr Siamak Salehi **CS**; Dr Greta Sawyer **CS**; Dr Sandra Strautnieks **LST**; Dr Robert Sutcliffe (Clinical) **LST #**; Dr Hector Vilca-Melendez (Clinical) **LST**; Dr Xiaohong Zhang (Clinical) **CS #**

RESEARCH ASSISTANTS

Jemima Adams **LST**; Chris Gonde **LST**; Sharon Lehec **LST**; Dr Ying Qiu **CS**; Rebecca Schofield **CS**; James Underhill **LST**; Min Zhao **D&E**

PHD STUDENTS

Massimo Bearzatto **D&E**; Dr Dimitrios Bogdanos **LST**; Jane Byrne **LST**; Helen Clarke **N**; James Dromey **D&E**; Dr Wayel Jassem (Clinical) **LST**; Arif Khwaja **N**; Dr Kristian Lobner **D&E**; Maria-Serena Longhi **LST**; Christopher Mulgrew **N**; Maria Neonaki **LST**; Eleanor Oatham **D&E**; Shideh Pouria **N**; Dr Paul Richardson (Clinical) **CS**; Dr Graham Roberts **D&E**; M Rosenthal **D&E**; Emma Taggart **LST**; Rachel Taylor **LST**; Claire Terry **LST**; Sarah Weenink **D&E**; Dr Xiaohong Zhang (Clinical) **CS**; Min Zhao **D&E**

MD/MS CANDIDATES

Dr Karen Anthony **D&E**; Dr Antony Antoniou **LST**; Dr William Bernal **LST**; Dr Emma Bingham **D&E**; Dr D Fowler **D&E**; Dr Jacqueline Gilbert **D&E**; Dr Rachel Harry **LST**; Dr Hemant Kocher **N**; Dr Rifat Malik **D&E**; Dr Ashraf Mikhail **N**; Dr Jonathan Mitchell **LST**; Dr James O'Beirne **LST**; Dr Martin Phillips **LST**; Dr R Salim **D&E**; Dr Diurmuid Smith **D&E**; Dr Robert Sutcliffe **LST**

RESEARCH NURSES

Sarah Hodgson **LST**; Julia Laurence **LST**; Ruby Maseon **LST**; Andrew Pernet **D&E**; Louise Taylor **LST**; Sarah Tizzard **LST**; Bula Wilson **D&E**

ACADEMIC ADMINISTRATION/PAs/SECRETARIES

Jackie Brooks **CS**; Eileen Withrington **LST**

cell and allogeneic haematopoetic stem cell transplantation. This project will link very well with programmes in the Institute of Psychiatry and the School of Biomedical Sciences.

Key research achievements

- Development of new techniques for isolation and cryopreservation of functional human hepatocytes.
- First successful hepatocyte transplantations worldwide for metabolic liver diseases in children using cryopreserved hepatocytes.
- First use of a single donor organ for three liver transplant recipients (right lobe for an adult, left lateral segment for a child, segment 4 for hepatocyte transplantation).
- Development of new techniques for isolation and preservation of functional pancreatic islets.
- First successful pancreatic islet cell transplantation in the UK for 20 years.
- Development of a highly sensitive radioligand immunoassay for anti-SLA antibodies for specific diagnosis of autoimmune hepatitis.
- CD4 epitope mapping of CYP2D6 the autoantigen of autoimmune hepatitis Type 2.
- Identification of cytochrome P4502D6$_{193\text{-}212}$ (CYP2D6$_{193\text{-}212}$) as a new immunodominant epitope of humoral immunity in Type 2 autoimmune hepatitis.
- First demonstration that molecular mimicry can be a cause of autoimmunity associated with chronic B and C viral hepatitis.
- First description of *de novo* autoimmune hepatitis (AIH) after liver transplantation for non-AIH disorders.
- First description of autoimmune sclerosing cholangitis in children and young adults.
- First cloning of the gene (*ABCB11*) for the human bile salt export pump (BSEP), the protein which is the main driving force for bile flow, and its expression *in vitro*.
- First demonstration that dendritic cells from patients with hepatitis C virus infection have an impaired ability to stimulate allo T cells.
- First clinical trial of tumour lysate-pulsed autologous dendritic cells for immunotherapy in hepatocellular carcinoma.
- First description that components of *E.coli* mimic the dominant epitope of the pyruvate dehydrogenase complex E2, the target of antimitochondrial antibodies in primary biliary cirrhosis, and are targets of cross-reactive immune responses.
- First demonstration that mimicry and cross-reactivity at the T cell level is required for autoantibody production against the major mitochondrial antigens in primary biliary cirrhosis.
- First report of the relationship between thiopurine methyltransferase genotype and phenotype and response to azathioprine therapy in autoimmune hepatitis.
- First demonstration of "immune paralysis" as a factor in severity of acute hepatic failure.
- First demonstration of insulin sensitivity of brain glucose metabolism *in vivo* in man.
- Demonstration that measurement of autoantibodies can predict remission from Type 1 diabetes in patients treated with immunosuppression.
- Demonstration that paracrine effects of insulin are important for beta cell maturation in early life.

Main research programmes

Cell Transplantation

- Isolation and cryopreservation of functional human hepatocytes for transplantation (Dhawan, Heaton, Hughes, Lehec, Mieli-Vergani, Mitry, Terry)
- Isolation and transplantation of functional human pancreatic islets (Amiel, Heaton, Huang)
- Development of insulin secreting cells from novel sources (hepatoytes, exocrine pancreas, beta cell culture) (Amiel, Huang, Dhawan)

- Genetic manipulation of human pancreatic cells to encourage growth (Amiel, Fabre, Huang)
- Genetic manipulation of isolated human hepatocytes to encourage engraftment after transplantation (Collins, Dhawan, Fabre, Hughes, Lehec, Mitry)
- The study of pancreatic development and the maturation of insulin secretory responses, generating data to support the development of α-cell surrogates for transplantation (Christie)

Liver Immunopathology and Endocrine Autoimmunity

- Definition of novel antigens and epitope targets of autoantibodies in autoimmune liver diseases and diabetes (Banga, Bogdanos, Christie, Ma, Vergani, Longhi)
- Immunohistochemical characterisation of inflammatory infiltrates in autoimmune and viral liver diseases (Hussain, Almerighi, Vergani, Portmann, Knisely)
- Role of immune paralysis in acute liver failure and end-stage liver disease (Antoniades, Vergani, Wendon)
- The role of vascular endothelial cells as antigen presenting cells during graft rejection following liver transplantation (Collins, Fabre, Zhang)
- The prediction and prevention of Type 1 diabetes and its recurrence post transplantation by antigen-specific immunotherapy (Amiel, Christie, Huang)

Cancer Immunotherapy

- Autologous dendritic cell vaccination for hepatocellular carcinoma (Daniels, Hannoe, Harrison, Mitchell)
- Characterisation of the effect of hepatitis C virus infection on dendritic cell function (Daniels, Hannoe, Harrison)

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- Identification of novel hepatocellular carcinoma tumour-associated genes (Daniels, Hannoe, Harrison)
- Evaluation of the interactions between allogeneic responses and tumour immunity (Fabre)

Molecular Genetics

- Molecular basis of cholestasis and bile formation (Byrne, Taggart, Thompson, Strautnieks)
- Phenotype-genotype comparisons in cholestatic liver disease and genotype influence on response to therapeutic intervention (Byrne, Taggart, Thompson, Strautnieks)
- Structure and function of novel genes in iron metabolism (Bomford, Neonaki)
- Nutrient-gene interactions in human and animal models of iron overload (Bomford, Neonaki)
- Genotype-phenotype correlations in haemochromatosis and the impact of modifier genes (Bomford, Neonaki)
- Investigation of polymorphisms in the genes encoding proteins which transport and metabolise immunosuppressive drugs and their effect on drug toxicity (Adams, Gonde, Hendry, Murphy, Sharpe, Tredger, Wendon)

Gene Therapy

- Development of a clinically applicable technique for non-viral gene delivery to the liver via the portal vein (Collins, Fabre, Zhang)
- *In vivo* gene delivery to the kidney, liver and pancreas in the context of transplantation and disease (Amiel, Collins, Dhawan, Fabre, Hendry, Huang, Hughes, Mitry, Sharpe)
- Definition of an optimal, clinically applicable technique for non-viral gene delivery to the corneal endothelium (Collins, Fabre)
- Ex vivo gene delivery to isolated hepatocytes (Collins, Dhawan, Fabre, Hughes, Mitry, Zhang)

Physiology and Metabolism

- Brain metabolism and function in health and disease (Amiel, Miell, Wendon)
- Role of the liver and its portal system in the control of metabolism (Amiel, Miell, Wendon)
- Investigations into the central control of peripheral metabolism using neuroimaging and pharmacological methodology (Amiel)
- Growth hormone insulin-like growth factor axis in the context of peripheral and hepatic insulin resistance in chronic liver disease and diabetes (Amiel, Miell, Wendon)
- Effect of different feeding regimens on the outcome of acute and chronic liver failure (Wendon)
- Hormonal and metabolic effects of insulin resistance in endocrine and hepatic disease (Miell, Amiel)
- Chronic effects of disorders of metabolism and their prevention (Amiel, with NHS collaborators, Drs Stephen Thomas and Michael Edmonds)

Teaching

The Division has a major commitment to undergraduate and postgraduate teaching. Dr Ian McFarlane is Deputy Academic Director of the Institute of Liver Studies at King's College Hospital Trust (KCHT).

Particular contributions of the Division are as follows:

- Development of the revised MBBS curriculum
- Leading in the MBBS SSM programme
- Contributing to intercalated MBBS and Biomedical School BSc courses
- Postgraduate training in particular skills associated with hepatology

Links with NHS and external bodies

Professor Stephanie Amiel

- Clinical Lead for KCHT Diabetes services
- Secretary of the Standing Advisory Committee in Diabetes and Endocrinology
- Member of the MRC's Physiological Medicine and Infections Board
- Member of the Steering Committee of the UK Dose Adjustment for Normal Eating (DAFNE) project
- Member of the Medical Advisory Panel on Diabetes and Driving to the Secretary of State and DVLA
- Regional Specialty Advisor South Thames (East) on Diabetes and Endocrinology
- Member of the Intercollegiate Working Party on Adolescent Medicine
- Founder member, DAFNE steering group

Dr Adrian Bomford

- Member of the UK Haemochromatosis Consortium

Dr Anil Dhawan (Honorary Senior Lecturer)

- Deputy Clinical Director of the Supraregional Paediatric Liver Service at KCHT
- Honorary Medical Director of the Children's Transplant Foundation

- Secretary of the Committee in Hepatology in the European Society of Paediatric Gastroenterology, Hepatology and Nutrition.
- Advisor on hepatology to the Royal Colleges Specialist Advisory Committee
- Member of the Equivalence subcommittee of the Royal College of Paediatrics and Child Health
- NHS Trust representative for South Thames at the Royal College of Paediatrics and Child Health

Professor John Fabre
- Expert Assessor for the UK Xenotransplantation Interim Regulatory Authority
- Member of the founding Executive of the British Society for Gene Therapy (BSGT)

Dr Nedim Hadzic
- Member of the scientific committee of The Alpha-1 Antitrypsin Deficiency International Research Group (AIR)

Dr Phillip Harrison
- Lead clinician for endoscopy at KCHT
- Lead clinician for viral hepatitis at KCHT
- Member hepatobiliary oncology multidisciplinary group for SE Thames region
- Member British Society of Gastroenterology ERCP National Audit Committee

Mr Nigel Heaton
- Head of the Liver Transplant Surgical Service at KCHT

Professor Bruce Hendry
- President of the Renal Section of the Royal Society of Medicine
- Member Program Committee of The American Society of Nephrology
- Member Program Committee of The American Heart Association

Dr Robin Hughes
- Project co-ordinator for the International Association for the Promotion of Cooperation with Scientists of the New Independent States of the Former Soviet Union (INTAS)
- Member of the International Hepatic Encephalopathy Group
- Member of the Liver Support Working Group of the European Society of Artificial Organs
- Member of the European Liver Cell Network
- Member of the Peer Review College of the Engineering and Physical Sciences Research Council

Dr Ian McFarlane
- Head of Laboratories, Institute of Liver Studies, KCHT

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A liver transplant

- Co-founder and Chairman of the International Autoimmune Hepatitis Group

Professor Giorgina Mieli-Vergani
- Clinical Director of the Supraregional Paediatric Liver Service
- Council member of the British Association for the Study of the Liver
- Co-founder and Paediatric representative of the International Autoimmune Hepatitis Group

Dr John Miell
- Clinical Director Acute and Emergency Medicine LUHT
- Head and Clinical Lead, IDEA Project, ESC
- Lead, Design Team, PFI build
- Consultant to the Scientific Advisory Board of DSL, Texas, USA

Mr Mohamed Rela
- Member of the UK Transplant Audit Committee
- Deputy Clinical Director of Adult Liver Services at KCHT

Dr J. Michael Tredger
- Head of Immunosuppressive Drug Monitoring Service for KCHT Liver Transplant Programme
- Member of the Review Panel of the National Kidney Research Fund

Professor Diego Vergani
- Director of the Alex Mowat liver immunopathology laboratory at KCHT
- Co-founder of the International Autoimmune Hepatitis Group and Chairman of its Serology subcommittee
- Liver Representative on the NIH (Bethesda, USA) International Workshop on Autoantibodies as Predictors of Autoimmune Disease

Dr Julia Wendon
- Clinical Director of Adult Liver Services at KCHT
- Head of Liver Intensive Therapy Unit at KCHT

Career progression of Divisional staff and recent appointments

The Division has been significantly strengthened by the appointment of Professor Diego Vergani to the Chair of Liver Immunopathology in the Department of Liver Studies & Transplantation. He has brought with him a team of highly experienced research staff who will make a major contribution to the Division's research on the immunopathology of autoimmune and viral liver diseases, and on tolerance, rejection and disease recurrence in liver transplantation.

Key publications

Byrne J A, Strautnieks S S, Mieli-Vergani G, Higgins C, Linton KJ, Thompson R J. 2002. The human bile salt export pump: Characterization of substrate specificity and identification of inhibitors. Gastroenterology: 123:1649-1658.

Clarke H C, Kocher H M, Kloog Y, Cook H T, Hendry B M. 2003. Ras antagonist farnesylthiosalicylic acid (FTS) reduces glomerular cellular proliferation and macrophage number in rat Thy-1 nephritis. Journal of the American Society of Nephrology: 14:848-854.

Crossey P A, Pillai C C, Miell J P. 2002. Altered placental development and intrauterine growth restriction in IGF binding protein-1 transgenic mice. Journal of Clinical Investigation: 110:411-418.

Galea-Lauri J, Darling D, Mufti G, Harrison P, Farzaneh F. 2002. Eliciting cytotoxic T lymphocytes against acute myeloid leukemia-derived antigens: evaluation of dendritic cell-leukemia cell hybrids and other antigen-loading strategies for dendritic cell-based

vaccination. Cancer Immunology & Immunotherapy: 51:299-310.

Harry R, Auzinger G, Wendon J. 2002. The clinical importance of adrenal insufficiency in acute hepatic dysfunction. Hepatology: 36:395-402.

Langley P G, Underhill J, Tredger J M, Norris S, McFarlane I G. 2002. Thiopurine methyltransferase phenotype and genotype in relation to azathioprine therapy in autoimmune hepatitis. Journal of Hepatology: 37:441-447.

Ma Y, Thomas M G, Okamoto M, Bogdanos D P, Nagl S, Kerkar N, Lopes A R, Muratori L, Lenzi M, Bianchi F B, Mieli-Vergani G, Vergani D. 2002. Key residues of a major cytochrome P4502D6 epitope are located on the surface of the molecule. Journal of Immunology: 169:277-85.

Wallace D F, Walker A P, Pietrangelo A, Clare M, Bomford A B, Dixon J L, Powell L W, Subramaniam V N, Dooley J S. 2002. Frequency of the S65C mutation of HFE and iron overload in 309 subjects heterozygous for C282Y. Journal of Hepatology: 36:474-479.

Zhang X, Sawyer G J, Dong X, Qiu Y, Collins L, Fabre J W. 2003. The *in vivo* use of chloroquine to promote non-viral gene delivery to the liver via the portal vein and bile duct. Journal of Gene Medicine: 5:209-218.

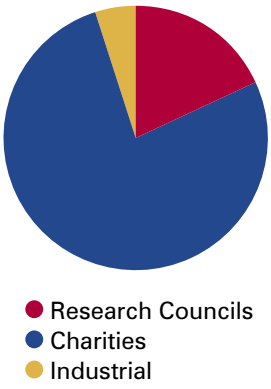
Zhao M, Christie M R, Heaton N, George S, Amiel S A, Huang G C. 2002. Amelioration of streptozotocin induced diabetic mice with human islet cells derived from long term culture *in vitro*. Transplantation: 73:1454-1460.

Prizes and awards

- Dr Claire Sharpe in Professor Hendry's group has been awarded a five year Department of Health/MRC Clinician Scientist Fellowship (£550,000) to continue her work on the role of Ras monomeric GTPases in epithelial-mesenchymal transdifferentiation in renal fibrosis.
- Dr Richard Thompson has received the Dame Sheila Sherlock Medal for hepatological research and has been nominated for the Hans Popper Award for the most significant scientific contribution to hepatology in the past three years. We congratulate them both on these achievements and wish them continued success in their work.

Research income

TOTAL VALUE: £5,840,000



In common with most other Divisions in the School, the Division of Hepatology & Transplantation derives research income support from a variety of sources including peer reviewed grants from research councils, government organisations, and charities, as well as some funding through collaborations with industry. Active grants during academic year 2002/03, totalling some £6 million, included:

Research Councils/Government
MRC, BBSRC, joint MRC/DoH, EU
TOTAL VALUE: £1,055,000

Charities
The Wellcome Trust, British Heart Foundation, Children's Liver Disease Foundation, Children Nationwide, Stanley Thomas Johnson Foundation, Golden Charitable Trust, Charles Wolfson Trust, Diabetes UK, Juvenile Diabetes Research Foundation, Diabetes Wellness & Research Foundation, Novo Nordisk Research Foundation, National Kidney Research Fund, GST Special Trustees, INTAS, Turners Support Society, The Royal Society, The Alex Mowat Trust, British Lung Foundation, KCHT/KCL Joint Research Committee, Association of International Cancer Research, ARC
TOTAL VALUE: £4,495,000

Industrial
Merck Sharpe & Dohme, Oxford Bioinnovations, Wyeth, Pfizer, DSL
TOTAL VALUE: £290,000

For further details of grants and publications please see Divisional web pages www.kcl.ac.uk/depsta/medicine/divisions/heptrans.html

IN FOCUS

Cell Transplantation

Hepatocyte Transplantation Team: L to R, Dr Ragai Mitry, Ms Sharon Lehec, Dr Robin Hughes, Dr Anil Dhawan and Ms Claire Terry.

Islet Transplantation Team: Front L to R, Ms Min Zhao, Ms Bula Wilson, Professor Stephanie Amiel, and Dr Emma Bingham. Back L to R, Dr Guo Cai Huang, Mr Andrew Pernet, Mr Joel Dunn and Dr Rifat Malik.

Laboratory studies

Transplantation of isolated cells as a therapeutic option for organ failure has several advantages over whole organ transplantation: it reduces reliance on donor organ availability, it "buys time" to allow the damaged organ to recover if possible or until a whole organ transplant can be performed if required, it allows for a wider range of donor tissue to be used, it avoids the need for major surgery, and the diseased organ can be left *in situ* for additional future therapies if required. Two major research programmes in the Division are devoted to studies on cell transplantation and its clinical application: hepatocyte transplantation (HTP), the

clinical lead for which is Dr Dhawan, and pancreatic islet cell transplantation (ITP) led by Professor Amiel. The two groups have jointly developed a purpose-built, aseptic, Good Manufacturing Practice (GMP) laboratory for isolation of cells for clinical use, and share interests in developmental aspects of cell therapy. The construction and operation of this facility has been made possible by generous financial support from the King's College Hospital Charitable Foundation, the Children's Liver Disease Foundation, the National Lotteries Community Fund, and the Dixons Charitable Foundation.

Initial studies focused on improving techniques for cell isolation and, in the case of hepatocytes, cryopreservation (to establish a hepatocyte bank). Both programmes have successfully produced very high yields of cells with greater than 90% viability and the human islet isolation programme (run by Dr Huang) is serving as the gold standard for work in the UK developing new islets from adult and embryonic stem cells and from adult exocrine tissue. In collaboration with Drs Jones and Persaud (School of Biomedical Sciences) these islets are being used for studies of human islet physiology and immunity.

The HTP group (Dhawan, Hughes, Lehec, Mitry, Terry) has been investigating the use of fetal organs and of organs from non-heart beating and other "marginal" donors as cell sources. The yield from fetal liver cells is relatively low but they have a greater stem cell potential to repopulate the liver. Collaboration with Drs Jones and Minger (School of Biomedical Sciences) as part of the KCL Stem Cell Consortium has been initiated to investigate their properties. Studies to determine whether transplanted hepatocytes persist and repopulate the liver are in progress, using immunochemical and molecular hybridisation techniques in explants from HTP patients subsequently receiving whole organ grafts to determine the location and characteristics of the donor hepatocytes. Engraftment of hepatocytes in the liver is dependent on cells entering the sinusoidal space and then being integrated into the liver structure. Initial studies on the use of growth factors to promote engraftment have shown promising results (Figure 1). In collaboration with Professor Fabre's group in the Division, who have successfully achieved *in vitro* gene transduction of isolated human hepatocytes using non-viral vectors, the application of *ex vivo* gene therapy to enhance growth factor(s) production in hepatocytes to stimulate cell engraftment is under investigation. Additional studies on cryopreservation, which is important for the long-term storage of cells for emergency clinical use, have shown that pre-incubation of hepatocytes with cytoprotectants, such as the antioxidant α-lipoic acid, before freezing improves their function on thawing (Figure 2). Hepatocytes are also being investigated as a potential source of glucose responsive insulin secreting cells, as are stem cells and non-endocrine pancreatic tissue.

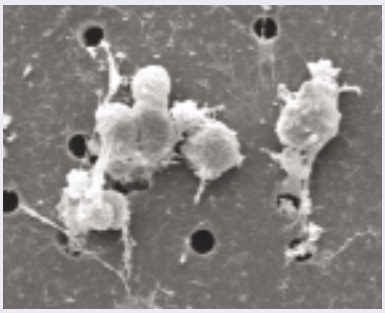


Figure 1: Scanning electron micrograph of human hepatocytes on a polycarbonate membrane in vitro. Cells are in the process of entering the pores following growth factor treatment (Mitry et al., to be published).

Clinical studies

During the year several cell transplants have been performed in patients with metabolic disorders. The first hepatocyte transplant was in a baby diagnosed antenatally with severe ornithine transcarbamylase deficiency, which is usually fatal within two weeks of birth. During the first three weeks of his life he received seven infusions of fresh and cryopreserved hepatocytes (total $1.8x10^9$ cells) through an umbilical vein catheter inserted at birth. A further infusion of $2x10^8$ cells was administered transhepatically at 5 months. Blood ammonia was maintained within acceptable limits throughout with additional pharmacological and dietary management. At 7 months the child underwent auxiliary liver transplantation with the aim of a complete cure of the urea cycle defect. A second child, a 3-month old boy with

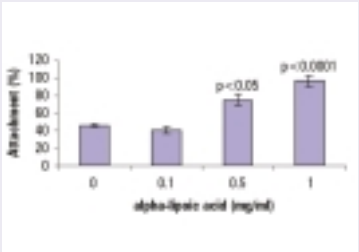


Figure 2: Effects of pre-incubation of rat hepatocytes with α-lipoic acid for 1.5h at 37°C prior to cryopreservation on cell attachment to plastic after thawing. Compared to no addition of α-lipoic acid (Terry et al., to be published).

congenital factor VII deficiency, received three infusions of cryopreserved hepatocytes over 5 days ($1.1x10^9$ cells) through an inferior mesenteric vein cannula. His requirement for recombinant factor VII infusion reduced by 80%. These are the first two successful human transplants with cryopreserved hepatocytes in the world. Recently, two children with progressive familial intrahepatic cholestasis type II each received a single infusion of fresh hepatocytes. In this condition the defect in the bile salt export pump rapidly leads to severe cholestatic liver disease. This is a new indication for HTP where there is a selective advantage for donor hepatocytes to repopulate the liver. Additionally, the first successful islet transplant in the UK for 20 years was performed in a patient with Type 1 diabetes, which to date has resulted in a reduction in her insulin requirement by 50%, normalisation of her HbA1c and abolition of her problematic hypoglycaemia.

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Mitry RR, Hughes RD, Dhawan A. 2002. Progress in human hepatocytes: isolation, culture and cryopreservation. Cell & Developmental Biology; 13: 463-467.

Mitry RR, Hughes RD, Aw MM, Terry C, Mieli-Vergani G, Girlanda R, Muiesan P, Rela M, Heaton ND, Dhawan A. 2003. Human hepatocyte isolation and relationship to early graft function. Cell Transplantation; 12: 69-74.