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Orthotopic liver transplantation in the making: key pioneers and events relevant to current practice

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Abstract

Within the field of liver transplantation the concepts of warm liver transplants and hepatocyte transplantation are currently under investigation. Although such novel research efforts are intricate and complex, the concept of human liver transplantation posed quite a challenge itself in the not so distant past. The development of the orthotopic liver transplant relied on numerous individuals and novel approaches of the time. Success resulted following cumulative developments in early experiments when Thomas Earl Starzl performed the first human liver transplantation 50 years ago in 1963. This article highlights key developments in the lead up to such an attempt, details of the procedure itself and biographies of three key leaders; Alexis Carrel, Peter Medawar, and Thomas Starzl.

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HISTORICAL

Liver transplantation in the 21st Century

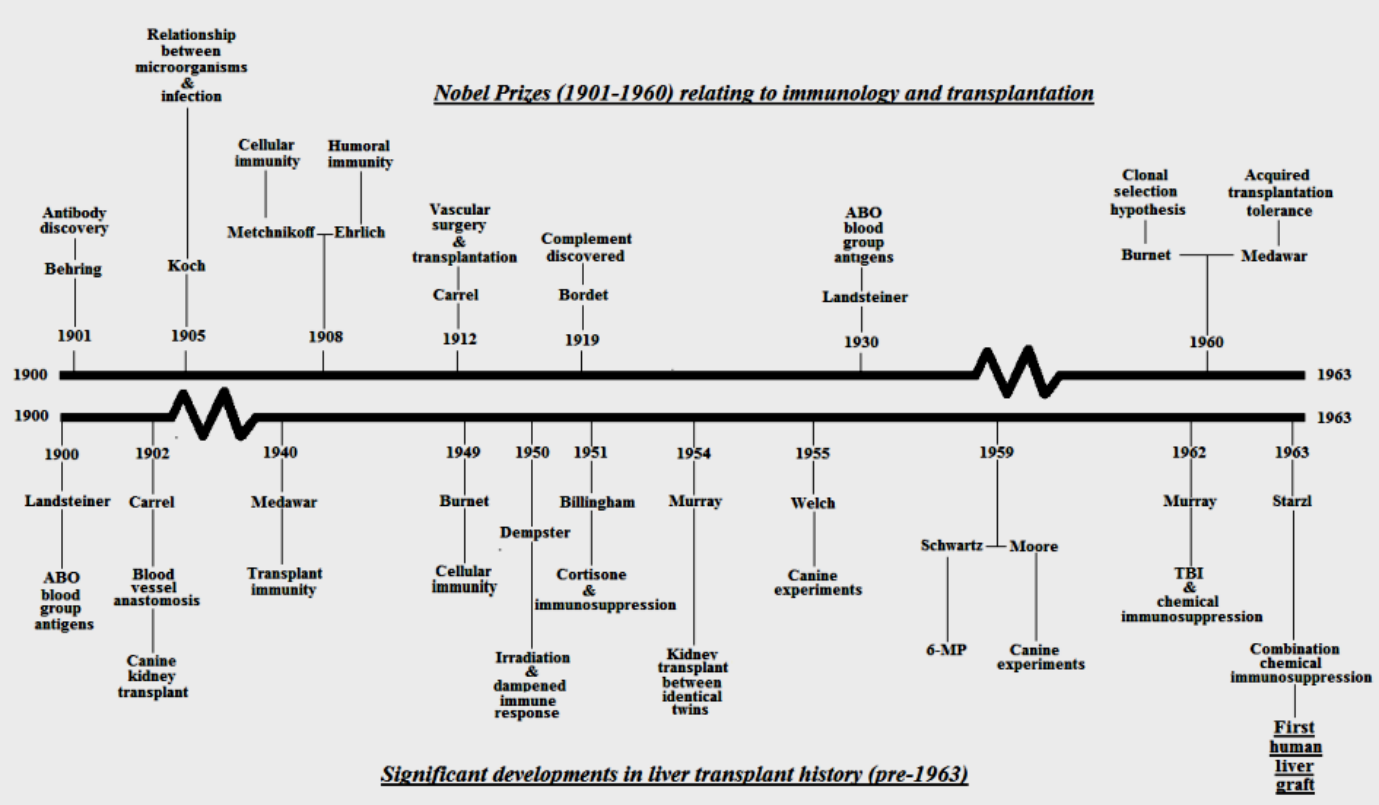
Orthotopic liver transplantation (OLT) (see Box 1) is currently the only curative treatment for patients with end-stage liver failure. Although quality and length of life can be improved, patients are committed to lifelong immunosuppressant therapy. This carries its own risks in addition to those associated with the procedure.

In the UK, at the end of March 2012, 553 of 7,636 patients on the active transplant list were in need of a new liver.[1] A 25% increase in liver-related deaths were reported between 2001 and 2009, where a male predominance was seen and the majority of cases occurred in those under 70 years old.[2] This signifies both the increasing burden on the NHS and the importance of OLT. As with many areas of organ transplantation, the demand far exceeds supply. 819 patients were removed from the total active waiting list during the financial year due to clinical deterioration and 508 died while waiting for a transplant.[1]

Although transplant research has an extensive history, liver transplant developments span over a much shorter period. The year 2013 signifies an important landmark in liver transplantation with the first human OLT taking place 50 years ago. The general principles discovered in the build-up to such a success are discussed within this article and many of the basic concepts can be applied across transplantation medicine. Although significant developments have been made over that time, problems with organ supply still need to be tackled; techniques need to be refined and new technologies researched. The recent use of 'warm liver' transplants by the University of Oxford has proved successful, and novel approaches such as hepatocyte transplantations are currently under investigation.[4,5]

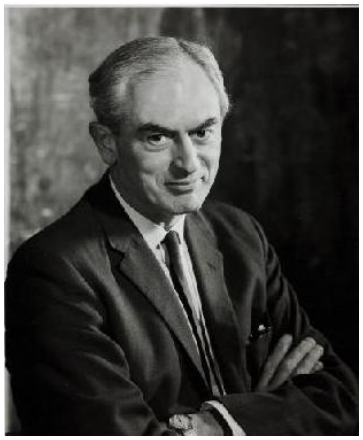
The history of liver transplantation (see Figure 1) is an expansive topic; the purpose of this article is to focus on the key developments that led to the first human liver transplant.

Box 1. Key terms within transplantation[3]
Orthotopic graft: tissue or organ transplant is placed in the same anatomical site as the original organ or tissue (e.g. heart or liver transplant)
Heterotopic graft: tissue or organ transplant is placed within a different anatomical site (e.g. kidney transplant)
Allograft (homograft): tissue or organ transplanted between genetically non-identical individuals of the same species (e.g. heart or liver transplants)
Autograft: tissue or organ transplanted between different sites of the same individual (e.g. skin transplants)
Heterograft (xenograft): tissue or organ transplanted from a different species (e.g. pig to human)
Isograft (syngraft): tissue or organ transplanted between genetically identical individuals



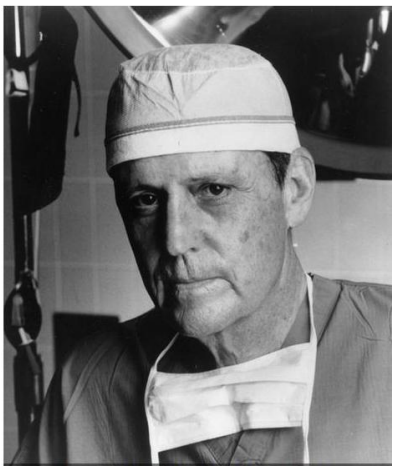
Alexis Carrel (1873-1944) Lyon, France

- Major in the French Army Medical Corp (1914-1919) where he developed new methods to treat war wounds
- Key developments include methods for blood vessel anastomoses (1902), whole organ transplantation (1908), and effective cold storage of blood vessels (1910).
- He was the youngest Nobel Laureate age 39



Peter Medawar (1915-1987) Rio de Janeiro, Brazil

- Early work focussed on regeneration of peripheral nerves, tissue culture and development of organisms
- Investigated skin grafts following requests from the Medical Research Council during the Second World War (1939-1945)
- Once elected fellow of the Royal Society, London, he was then awarded a Royal Medal (1959) and Nobel Prize (1960)



Thomas Earl Starzl (1926-), Iowa

- Post-graduate surgical training focussed on vascular, thoracic and general surgery
- Extensive research in organ transplantation with over 1600 publications
- Performed the first human liver transplant (1963)

Figure 1. Key pioneers[6-8], Nobel Prizes (1901 – 1960) relating to immunology and transplantation[9], and significant developments in liver transplantation history (pre-1963). *6-MP: 6-mercaptopurine, TBI: total body irradiation*. Alexis Carrel and Peter Medawar images courtesy of the Wellcome Library, London. Thomas Starzl image courtesy of the Archives Service Center, University of Pittsburgh.

Transplant immunology

Transplant immunology: blood reactions

Karl Landsteiner, an assistant within the Pathological-Anatomical Institute in Vienna, was the first to discover blood groups in 1900 following agglutination reactions, whereby antibodies and red blood cells, or corpuscles, adhere to form a mass, in normal human serum.[10] Originally believed to only occur in diseased states, the reaction was later found in 22 healthy individuals, which categorized individuals into blood groups A, B or C.[10] The possibility of successful blood transfusions became a reality. Identification of these blood groups was one of the first key steps in transplantation: correct identification and matching of individuals based on their blood group is a necessity in transplantations to prevent the agglutination process that could result in transplant failure.

Transplant immunology: the mid-20th century

Following on from Landsteiner, Medawar found, while working for the Medical Research Council in the 1940s, tissue reactions when using skin grafts.[11] Skin homografts from the patient's brother were used in a 22 year old woman with extensive burns. Subsequent biopsy specimens during each dressing change allowed the determination of local reactions within the tissues.[11] At day five, both sets of grafts were similar to the naked eye and histologically.[11] A subsequent polymorph and lymphocyte infiltration of collagen, and a multinucleate giant cell reaction, were seen.[11] By day fifteen retrogressive changes had been shown, involving both a mesenchymal and vascular reaction, with a thin detached band of skin now replacing the previously well-grown tissue.[11] Medawar reported the destruction of foreign tissue as the result of an antigen-antibody reaction.[11] In 1949 Burnet also showed how, when exposed to foreign antigens, antibodies were produced and further work developed the concept of cellular immunity.[12] The immune system would target foreign body cells and cause destruction of tissue. Whether it was immediate or within weeks, the transplantation of organs between non-identical humans would eventually resulted in rejection. A second culprit for the rejection reaction was identified. Compatible blood groups and tissue matching, a much more intricate task, were necessities for transplantation. Even so, a lack of control over the immune system was responsible for many of the earlier failures.

Transplant immunology: early findings of tissue rejection

The immune system needed to be suppressed after transplantation, and interest built in the use of cortisone and total body irradiation in an attempt to dampen down the immune response.

In 1951, Billingham's work with skin homografts and autografts in rabbits showed a role for cortisone in preventing transplant rejection.[13] The transplanted tissues became swollen and hard, with a colour change to brick red and subsequently to what is described as "shades of dirty yellow, green or brown".[13] The tissue eventually degraded. Interestingly, following administration of cortisone it was reported that the usual acute progressive rejection, comprising of an inflammatory response, oedematous swelling

and lymphocytic infiltration, was converted to a chronic delayed reaction with intermittent progress.[13] Cortisone extended the life of the homograft, with the belief that it either reduces the ability of the graft to elicit a response or by causing a direct dampening of the response itself.[13]

Around the same time, kidney transplants were also being tested. Donor kidneys were being used from those who had succumbed to the guillotine and, although urine production was observed initially, the end result was unsuccessful in unmodified recipients.[12] It would be three more years until a live donor kidney transplant took place between a mother and son. A mere three week survival of the graft was seen in another unmodified recipient.[12]

Transplant immunology: dampening down the immune response

As irradiation had already been proven effective at suppressing antibody production towards bacteria, Dempster wanted to apply irradiation to transplantation, reporting his experiments in 1950.[14] Skin homografts in rabbits were utilized and irradiation was given at an average dose of 250 roentgens, resulting in prolonged survival of the grafts.[14] Although successful at dampening the immune response, the species used were resistant to irradiation of this type.[14] It was believed such a therapy was inapplicable to humans, but the fact remained that the rejection reaction was modifiable. Further work was required to develop more appropriate techniques to tackle the immune system.

Significant findings in transplant immunology, such as tolerance in mice with immature immune systems, were later discovered by the Bilingham-Brent-Medawar experiments.[12] Attention moved towards bone marrow and a form of tolerance was identified if recipients also had donor leukocytes in circulation. Nevertheless, unless tissues are closely matched, immunocompetent donor leukocytes hold the potential to lead to a graft-versus-host disease.

Transplant immunology: genetic makeup

A key landmark, and one of the first real successes of the time, came when Dr Joseph Murray and his team transplanted a kidney between identical twin brothers in 1954.[15] Prior to this, unsuccessful attempts had been made in Russia and the United States of America using both canines and humans.[15] Although the grafts initially showed success with urine output, urine production eventually ceased following a period of haematuria.[15] At the time, 9 kidney transplants had been attempted by Murray's group. Four showed significant renal function, with one showing clinical improvement for 5 months.[15] All were eventually unsuccessful. It had already been shown that skin grafts between identical twins lacked the rejection reaction. Through reciprocal skin grafting, the two individuals under question were identified as identical.[15] The need for kidney transplantation followed a history of chronic glomerulonephritis in one twin with cardiomegaly, raised blood pressure and oedema.[15] Following the procedure these normalised but, after discharge, urine showed casts and blood pressure rose.[15] Removal of diseased kidneys eradicated these problems and the graft was successful for 25 years.[12] Key problems in kidney transplantation seemed to revolve around blood grouping, immune responses and disease status of the organ.[15] Elective donors

over cadavers and intra-abdominal placement of the kidney were preferred.[15] The group also report infection playing a significant role in the destruction of donor tissue.[15]

Transplant immunology: back to irradiation

Although already identified as a potentially harmful modality, total body irradiation was finally applied to humans in 1962 where Murray et al. described experiences with the use of both chemical immunosuppression and irradiation.[16] Out of twelve selected for total body irradiation (TBI) only one survived: a patient who received a kidney from a dizygotic twin.[16] Murray also notes a total of 27 failed transplants using irradiation.[16] With regards to chemical immunosuppression, thiopurine and actinomycin C were under investigation and, although 5 of 6 developed measurable renal function, only 1 survived over 120 days.[16] Research was moving away from radiation and moving towards new pharmaceutical developments.

Transplant immunology: novel components

With developments in chemical immunosuppression, thoughts focused on an antimetabolite, 6-mercaptopurine (6-MP). Success had been found in reducing antibody response in rats and so Schwartz in 1959 carried out experiments on rabbits, again making use of homografts.[17] 4 groups were treated with 6-MP at varying doses for similar time periods. Mean rejection time increased in correlation with higher 6-MP doses and, although similar doses were used between one of the subcutaneous and oral groups, the oral route was found to be less effective at prolonging rejection time.[17] Following the initial homografts, the rabbits were given second homografts and a uniform accelerated rejection was seen.[17] Chemical immunosuppression alone had proved to be effective.

Combinations of drugs were then used by Thomas Earl Starzl in 1963.[18] He experimented with the use of prednisone in addition to azathioprine in human renal transplantation. Azathioprine, a derivative of 6-mercaptopurine, was the mainstay treatment prior to the operation and was continued in all cases unless agranulocytosis persisted.[18] At the time of rejection doses were increased with the hope of preventing the process. If unsuccessful, prednisone, 150-400mg daily, was used secondarily as an antirejection therapy.[18] The spleen and thyroid were removed in the majority of cases and intravenous actinomycin C administered during a rejection crisis.[18] The combination was found to be successful at reversing rejection crises and preventing secondary rejection attempts.[18] It had been shown that individuals tolerated grafts in the setting of chemical immunosuppression.

Technical aspects to transplantation

In the 19th century it had been impossible to repair blood vessels and early experiments with other organs had failed. Transplanting the liver, a complex organ with a dual blood supply, posed quite a challenge and technical advances were needed. Research focused mainly on the skin and kidney in subhuman species, ranging from rats and rabbits to canines. A major challenge of the time was tissue rejection; regarding both blood groups and tissue matching.

Basic technical aspects: blood vessel repair

Aside from identifying and tackling problems of tissue rejection, the challenging technical components to transplantation also needed to be considered. At the time, tissues could be repaired but there were no means to successfully transfer organs within an individual, let alone between individuals. At the start of the 20th century research focused on surgical techniques and continued to make use of subhuman species.[12] Alexis Carrel's work in 1902 opened the door to organ transplantation.[9] He aimed to refine anastomosis techniques in order to allow for blood vessels to be repaired just like other tissues were at the time. This is essential for transplantation as without adequate blood supply the graft will fail with necrosis of the involved tissues.

Carrel believed by improving techniques, lives such as that of the French president Sadi Carnot, stabbed in 1894, could be saved.[9] Using finer needles, developing the triangular method of anastomosis and demonstrating, with the help of Charles Guthrie, that veins could be used as arteries gave promise to the idea of transplantation.[9] Carrel successfully transplanted a dog's kidney to its neck from its abdomen in 1902.[9]

Technical aspects: the build-up to the orthotopic liver transplant

An effective procedure for the placement of axillary whole livers with results of around twelve days survival had been shown by Moore and Welch between 1955-1959.[19,20] Starzl used this approach in his own experiments with canine species and found an improved mortality rate.[21,22,23] Technical failures were likely, and prolonged survival with the use of azathioprine, along with its hepatotoxic effects, were reported.[23] It had become evident that vascularization was key in survival of grafts. Damage to the vasculature needed to be avoided as did any potential ischaemic damage to donor organs. Hypothermia, within the range of 10-20°C, protected the liver for a short period, but prolonged ischaemia eventually resulted in liver injury.[21] Similarly, excessive venous flow was also reported as damaging, resulting in congestion.[21] The longest survival seen with this early work was 20.5 days. Multi-organ transplants were also considered as fewer vascular anastomoses are required but success rates were low.[22] Congestion and haemorrhage were reported as the leading causes of death.[22]

Following breakthroughs with anti-rejection therapy and the development of a successful transplant technique in canines, the opportunity arose for attempts in humans. Starzl set the stage for the orthotopic technique used today with the world's first human liver graft in 1963.[24]

Orthotopic liver transplant: the procedure

At the time, successful liver transplantation would allow a major leap forward in the treatment of primary hepatoma, congenital atresia of the biliary system and terminal cirrhosis, for example.[24] Details of the procedure, many of which remain today, can be found in Starzl's original article.[24]

One key step within Starzl's procedure was the use of hypothermic perfusion. Early research had shown variable tissue sensitivity to anoxia which, if prolonged, could result in irreversible cellular injury. As of such Starzl used 5% dextrose, penicillin, heparin and

procaine hydrochloride, precooled to 15°C to perfuse the donor liver in Starzl's transplant. The donor body was also initially cooled to 15 °C, but increased to 20°C between 45 and 104 minutes later. With hypothermic perfusion the aim was to keep the donor liver viable for as long as possible yet previous work had shown hepatocellular injury results if such perfusion persists for more than 2 hours.

Although the operation was a success for two of the three subjects, survival was limited to 7.5 and 22 days. A number of problems were noted. The health of the donor organ was paramount; damage may lead to failure of the transplant and hypothermic perfusion for over 2 hours was detrimental. During the operation, increased fibrinolytic activity was noted close to the time of manipulation and removal of the liver, causing a bleeding diathesis in the first patient, and a hypercoagulable state followed, with pulmonary emboli present in the final two patients on autopsy. The need for improved coagulation control was documented.[24]

From vessel repair to human liver transplants

With further refinement of the technique and drug development, the procedure became more successful and at present 86% of transplanted livers in the UK function well within the first year.[25] Initial work by both Landsteiner and Medawar brought to light the importance of the immune system in causing tissue reactions, and ultimately the cause of transplant failure that was seen throughout early experiments. Dating back as far as 1902, without Carrel's work on vascular anastomoses Starzl's work would have been unapproachable. The use of subhuman species, specifically canines, built the technical success which made Starzl's procedure possible. With regards to current practice, knowledge can be gained even from the earliest attempts at OLT with the use of hypothermic perfusion and improved coagulation control, for example. Understanding the history of OLT and the challenges that have been overcome identifies not only important concepts which have been established but also the pitfalls that can be prevented in future use. Forgotten advances may even shed light on future conundrums within transplantation and other specialties. Although the history of liver transplantation is not made up wholly of Nobel Laureates, a vast number of pioneers from very different background played key roles in the gradual advancements which have resulted in success of the once questioned idea, and without them the lives of many would have been lost.

Key Learning Points

- Prevalence rates and the demand for liver transplantation is rising
- The development of the orthotopic liver transplant relied on numerous individuals and novel approaches of the time. Success resulted following cumulative developments.
- Chemical immunosuppression is the mainstay treatment to control the immune system in liver transplantation but alternative approaches exist and may be of use if future challenges arise.
- Although key elements in the pre-1963 history of the orthotopic liver transplantation have been noted, the topic is extensive and numerous advances have been made in the past 50 years.

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