Cell transplantation

Overcoming Cell Rejection

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The transplantation of adult or embryonic cells offers high hopes in the treatment of diabetes, burns or neurological diseases. But why is progress so slow?

Cell transplantation remains a clinical experiment, despite the desperate need to be able to transfer different cells in order to treat various diseases. Such potential transfers include (to name just a few): myoblasts in muscle diseases, neural cells in neurological diseases and neuron damage, keratinocytes in skin burns, Langerhans islets in diabetes millitus and hepatocytes (HCs) in metabolic diseases. Such transplantation is also required for "bridging" terminal liver failure in patients waiting to receive liver transplants.

The transplantation of adult differentiated or embryonic cells gives us hope that we will be able to achieve the formation of tissues or organs *in vitro* or even *in vivo*. Isolating cells from organ or tissue parenchyma is a relatively easy procedure. Moreover, culturing isolated cells *in vitro* for long periods of time without loss of function is a routine procedure. Why, then, do morphologically intact and functional cells returned to their host organism and to their tissue of origin undergo rapid destruction, leading to so-called "early graft dysfunction"? When cells are transplanted to sites remote from their site of origin, the process of elimination progresses even more rapidly. Among millions of transplanted cells, only a few survive. What is the mechanism behind this elimination? What determines which cells survive, and what are their genetic and phenotypic properties? These are problems that we at the Medical Research Center are trying to solve. Our research on hepatocytes (liver cells) sheds light on several areas that may lead to progress in cell transplantation.

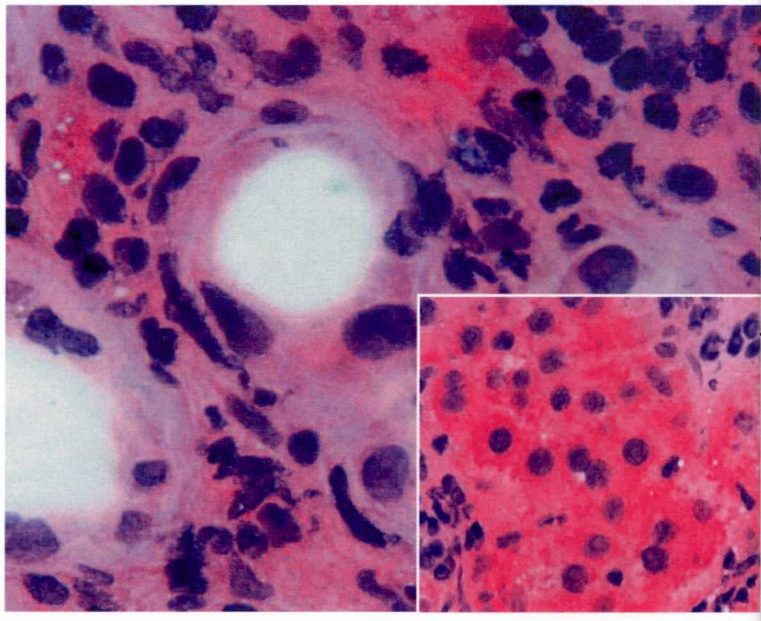
Touch or die

Anoikis is a process that develops in hepatocytes and other cells after they are isolated from their home organ or tissue, leading eventually to apoptosis – i.e. "cell suicide". Anoikis may be induced by the disruption of "survival signals" that are normally received by a cell attached to an extracellular matrix. However, growth factors can activate pathways that confer resistance to anoikis. HGF (hepatocyte growth factor) is able to protect cells not only from anoikis but also from apoptosis induced by a variety of injuries. In our studies, apoptosis of hepatocytes did not exceed 5% of cells 2 to 4 hours after isolation. Longer *in vitro* observations are needed to deepen our knowledge of anoikis and – prospectively – to counteract this process.

But the danger lies not only within the transplanted cells themselves, but also in the innate immune system engaged in mammalian tissue or graft cell recognition. Implanting any cell into tissue or organ parenchyma produces local cell and capillary damage. A surgical wound is created, with the immediate mobilization of platelets and granulocytes as well as the accumulation of clotting factors. The scavenging phase of wound healing begins.

Transplanted cells fall victim to the accumulation of host cells and humoral factors. We have studied the process of HC transplant elimination in portal and pulmonary veins, liver tissue, under the kidney capsule and

3 months after implantation, hepatocytes are doing fine in the spleen (small photo) – they are even starting to produce bile canuli (large photo)



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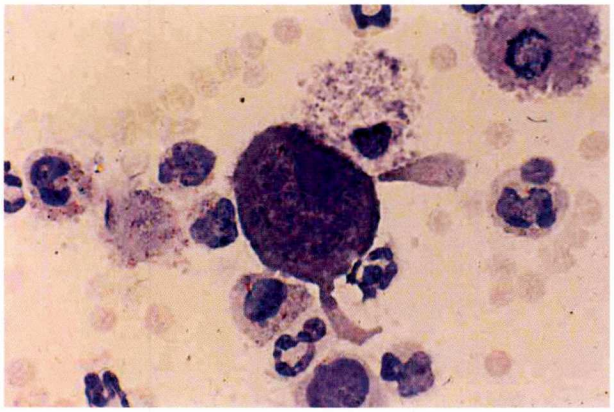

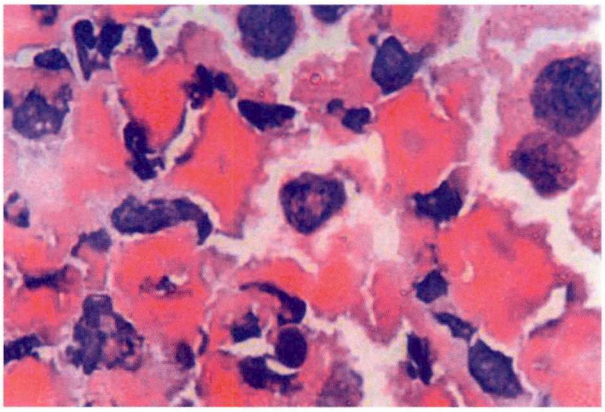

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Hepatocytes transplanted to any tissue other than the liver induce an attack by immune system cells, like macrophages or leukocytes. Only a few cells per million survive locally, irrespective of whether they were "strangers" of the recipient's own

in the peritoneum and subcutaneous tissue. Only a few cells per million survive locally, irrespective of whether they were "strangers" or the recipient's own.

Intravenously transplanted cells stand no chance of survival. The intravenous infusion of isolated HCs causes them to lodge in venules, with the immediate aggregation of platelets and granulocytes, a picture that is seen in both the lungs and portal vein tributaries. Intraperitoneal injection of HCs causes the fast mobilization of macrophages, which attach to the transplanted cells causing them to dissolve. Subcutaneous implantation of HCs is followed by an immediate mobilization of granulocytes and monocytes. Granulocytes marginate in vessels adjacent to the engraftment site within 1 hour.

Increasing the survival rate

The elimination of blood granulocytes and monocytes by non-lethal whole-body irradiation increases the survival rate of intravenously transplanted HCs. Non-lysed HCs may be found in the lungs and spleen hours later. We have recently developed a protocol enabling a large number of HCs transplanted into rat spleen to survive, allowing for the proliferation and formation of bile canaliculi. This protocol is based on the irradiation of the recipient with 8 Gy, the injection of an antiserum on the following day, the intravenous infusion of 10^7 syngeneic bone marrow cells on third day, a simultaneous intrasplenic injection of 10^7 HCs, and 3 consecutive partial hepatectomies at 3-week intervals. The follow-up was 90 days. In some cases, when HCs were engrafted under the splenic capsule, irregular liver lobules were formed. Other animals showed a large island of normal, dividing HCs, adjacent to newly formed bile canaliculi. Microscopic pictures were dominated by a large number of distended small bile ducts. The fragments of spleen with proliferating HCs and cholangiocytes showed the external appearance of a normal liver fragment. The role of oval cells in ectopic liver tissue formation in an individual with reconstituted bone marrow is now being studied.

Future of cell transplantation

Several conditions need to be met to achieve further progress in hepatocyte and other cell transplantation. Researchers and medics have to overcome detached-cell "suicide" (apoptosis), and restrain the innate immune reaction against transplanted cells. We still require more knowledge about the local cell chemical environment and signaling, and the regulation of DNA synthesis in transplanted cells (cell expansion). Further research is needed to better transfer genes of interest into cells using retrovirial carriers, as well as research on adult tissue-specific stem cells. If we master these areas and solve these problems, we will enter a new era of immunology - the immunology of individual organ cells and their environments. ■

Further reading:

Olszewski WL, Rudowska A, Mecner B, et al (2002). Autologous transplanted hepatocytes - lysis by leukocytes in vivo and in vitro. *Transplantation Proceedings* 34, 705.

Interewicz B, Maksymowicz M, Szyper E, et al (2002). Donor DNA from rejecting grafts is found in recipient tissues. *Transplantation Proceedings* 34, 699.

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The flow cytometer is a basic tool used to sort and identify cells prior to transplantation