New concepts in medicine

The role of cell migration and microchimerism in the induction of tolerance after solid organ transplantation

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Summary

A new hypothesis has been proposed which states that microchimerism is the basis for the clinical tolerance seen in long-term survivors of solid organ transplants. Efforts to enhance microchimerism include simultaneous infusion of bone marrow of donor origin and transplantation of a solid organ. Studies are in progress to verify the phenomenon of microchimerism and its role in clinical tolerance.

Keywords: microchimerism

The ultimate goal in transplantation is to promote the development of donor-specific immunologic unresponsiveness (tolerance), such that immunosuppressants are not required. The development of potent immunosuppressive agents has led to an increased incidence of complications. Therefore, identification of strategies for the induction of tolerance in clinical transplantation is highly desirable.

To achieve this state of tolerance, a variety of techniques such as radiotherapy, polyclonal or monoclonal sera, bone marrow infusion, and nonspecific immunosuppressive agents, have been tried with some success. However, the mechanisms underlying tolerance remain a subject of much debate. A number of theories have been proposed, including clonal deletion of antidonor cytotoxic T-cell precursors in the recipient, peripheral anergy by silencing of the IL-2 (interleukin-2) gene, suppression of alloreactive clonotypes, and clonal paralysis of alloreactive cells by donor 'veto cells'. However, a unified hypothesis has not emerged.^{1,2}

The potential of pretransplant blood transfusion in inducing tolerance has been well documented, but this is a nonspecific approach and may result in sensitivity, precluding a later transplant.³ Monoclonal antibodies such as anti-CD4 have been used to achieve tolerance in a variety of animal models.⁴ Recombinant cytokines such as IL-4 in conjunction with donor-specific transfusion and cyclosporin prolonged survival in the rat heart allograft model.⁵ A particularly novel strategy was to slant the immune response towards Th2 (T lymphocyte) and away from a Th1 response by the addition of IL-4 *in vitro*.⁶

The role of 'veto cells' in the induction of tolerance has been the subject of much debate.⁷ Some investigators have suggested that major histocompatibility complex (MHC) class I reactive cells are functionally deleted (vetoed) through interactions with the target cells expressing the specific epitope. However, other investigators found that the induction of tolerance to MHC class II antigens was independent of the veto phenomenon.⁸

Complications of immunosuppressive agents

It has been known for some time that chronic immunosuppression has numerous disadvantages. It can lead to fatal lymphoproliferative disease, increased incidence of cardiovascular mortality, diabetes mellitus, hyperlipidemia, hypertension, and chronic nephropathy.⁹ Investigators have attempted to reduce the complications of immunosuppressive therapy by lowering or withdrawing steroids, which has often resulted in an increased incidence of rejection episodes.⁹ It has been speculated that the timing of steroid reduction or withdrawal is critical, as late withdrawal has been shown to be better than earlier withdrawal. It has been proposed that those recipients of solid organ transplants who show increased levels of circulating donor cells may be candidates for early steroid reduction, and eventually complete withdrawal of immunosuppressants.^{10–16} Work in our laboratory is directed towards this goal.

Microchimerism: a new concept in graft tolerance

It was recently proposed by Starzl *et al*^{10–12} from the University of Pittsburgh that the exchange of migratory leukocytes between the organ transplant and the recipient led to the development of long-term tolerance. This phenomenon has been called microchimerism. The hypothesis is that the presence of donor (microchimeric) cells in recipients of organ transplants correlates with tolerance, and allows for the withdrawal or reduction of immunosuppression.

It was initially shown in female recipients of liver transplants from male cadaveric donors that parts of the macrophage system of the transplanted organ

Proposed mechanisms of immunological tolerance³⁹

- clonal deletion
- suppressor T cells
- anergy
- 'veto' cells
- microchimerism

Box 1

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Accepted 17 April 1996

were replaced by the cells of the female recipient (identified by the Barr bodies). Since then, there have been a flurry of reports showing that this form of cell migration from the host into the donor organs is a common occurrence.¹¹ This unique form of cell migration from the host-to-donor and *vice versa* (microchimerism) is thought to have important consequences in transplantation. It was proposed that the phenomenon of cell migration could cause accelerated rejection, graft-versus-host-disease (GVHD), or may be the basis of tolerance. On the other hand, it is entirely possible that these cells may simply be innocent bystanders.¹²

The subject of microchimerism and graft tolerance is currently under intense investigation. A number of factors may be involved in this process, such as the quality of immunosuppression, the quantity and quality of immunocytes in the organs, donor-recipient histocompatibility and as yet undefined factors.¹² It has also been shown that the donor cells departing from the solid organ graft and the recipient cells entering it are dendritic cells.^{13,14} Enhancement of microchimerism, such as by infusion of donor bone marrow, may lead to tolerance, but it could also have deleterious consequences, such as GVHD, or precipitation of acute allograft rejection.^{15,16} The fact that even six-antigen-matched kidneys have only a small advantage,¹⁷ may perhaps be explained by the phenomenon of microchimerism, whereby tolerance is due to microchimerism, and not to better HLA matching.¹⁶

A number of factors have been proposed to explain the coexistence of donor and recipient cells in the transplanted patient. These include a variety of cytokines, growth factors, and soluble class I antigens.

Enhancement of chimerism and tolerance by bone marrow infusion

It has been proposed that microchimerism may be enhanced by the perioperative infusion of bone marrow from the donor. Monaco *et al*¹⁸ showed that bone marrow could be a potent tolerogenic agent in recipients of solid organ transplants. What has been described as the 'Monaco model' uses antilymphocyte serum for induction of immunosuppression after infusion of cryopreserved cells after the primary allograft. These findings have been confirmed by others,^{19,20} who have suggested that the induction of tolerance was due to the surviving leukocytes behaving as veto cells.

Clinical trials aimed at enhancing microchimerism by infusion of bone marrow have had mixed results. Workers from the University of Alabama²¹ could demonstrate the presence of mixed allogeneic chimerism in kidney transplant recipients. However, this form of treatment did not result in a significant increase in rejection-free graft survival. Other investigators have failed to detect microchimerism when the liver transplant recipients were given donor bone marrow, despite using a variety of sensitive detection techniques such as polymerase chain reaction.²² The infusion of bone marrow did not have any adverse effects on the primary transplanted organ.

Investigators from the University of Pittsburgh²³ have reported the results of clinical trials in which they attempted to augment chimerism by infusion of donor bone marrow. Of the 18 patients reported (10 kidney, 7 liver and 1 heart recipient), they detected the presence of microchimerism in 17 patients, while GVHD was not of clinical significance. The degree of microchimerism was 1000-fold greater than in control patients, although immunosuppression could not be withdrawn in any patient. They later reported another group of kidney transplant recipients who underwent a combined kidney – bone marrow transplantation. They confirmed their previous results that stable chimerism could be regularly achieved; there was no GVHD, however, the addition of bone marrow did not influence graft function.²⁴

Microchimerism in relation to bone marrow transplantation

GVHD causes serious morbidity and even death in recipients of bone marrow transplantation. Recipients of bone marrow transplantation who suffer from GVHD show the presence of donor type cells in the recipient organs, particularly in the skin, intestinal mucosa, and the bone marrow. It was therefore postulated that the basis of GVHD was the persistence of donor cells in the recipient.

Attempts to enhance microchimerism in organ transplantation may conceivably cause deleterious side-effects. Indeed, some recipients of liver allografts have shown signs and symptoms of GVHD,^{25,26} but is rarely of clinical significance. In clinical trials when simultaneous bone marrow and solid organ transplantation was used to enhance microchimerism, GVHD has not reported to be a problem.^{21–24}

Techniques for detecting donor-type cells in the transplant recipient

- fluorescent *in-situ* hybridization or polymerase chain reaction to detect the presence of the Y chromosome in the recipient in case of sex-mismatched transplants
- polymerase chain reaction to detect the presence of donor DNA in the recipient by microsatellite analysis or to detect the presence of donor genotypes by SSOP analysis of the HLA-DRB1 region
- detection of donor-type MHC in the recipient in tissue sections based on immunohistology using a variety of monoclonal antibodies

Box 2

Techniques to detect cells of donor origin in the transplant recipient

A variety of techniques, based on chromosome analysis, polymerase chain reaction, and immunohistochemistry have been used to detect donor-specific cells in organ transplant recipients. The most widely used techniques are outlined below.

ANALYSIS OF Y CHROMOSOME

In sex-mismatched transplant cases (male donor, female recipient), DNA from the recipient is analysed by polymerase chain reaction, using X and Y chromosome-specific primers.²⁷ To minimise contamination, blood and DNA samples are processed by female laboratory staff. Fluorescent *in situ* hybridisation may also be used for this purpose. Chimerism in male recipients of female donors may be detected by the presence of Barr bodies.

Nagler *et al*²⁸ were able to detect systemic chimerism in peripheral lymphocytes at a level of 2–5% following sex-mismatched liver allografts in patients who did not experience rejection, but no donor cells were detected in patients who had clinical episodes of cellular rejection. Ninova *et al*²⁹ from the Mayo Clinic, also confirmed the utility of the fluorescent *in situ* hybridisation technique in detecting microchimerism, which was present in 27% of their sex-mismatched liver transplant recipients. The level of donor-type cells was significantly higher in the early posttransplant period; higher level of microchimerism was not, however, associated with an increased incidence of graft rejection.

Work in our laboratory has shown that X-Y chromosome DNA can be readily detected by polymerase chain reaction. We used polymerase chain reaction primers to amplify a portion of the X-Y homologous region, so that two bands were obtained from male DNA and a single band from female DNA. This procedure also acted as an internal control for polymerase chain reaction amplification. Blood samples were obtained from female and male donors and white cells isolated in the usual manner. Different quantities of male cells were added to a fixed quantity (1×10^5) of female cells, such that the percentage of male cells ranged from 0 to 10%. Approximately 1% of male DNA was readily detectable by gel electrophoresis and ethidium bromide staining of the polymerase chain reaction product. We could increase the sensitivity of this technique by including radioactively labeled dCTP in the reaction mixture.

MICROSATELLITE REPEAT POLYMORPHISMS

Microsatellite sequences (eg, CA repeats) are abundantly interspersed, repetitive DNA elements in the genome of many eukaryotes including humans. These microsatellites are a source of highly informative polymerase chain reaction-based genetic markers that appear to be evenly distributed throughout the human genome.³⁰ They have been found to be useful for distinguishing mixed cell populations. The appropriate region is amplified by polymerase chain reaction in the presence of P³²-labeled dCTP; the amplified DNA is denatured, and then electrophoresed on a denaturing polyacrylamide gel. The gel is dried and exposed to X-ray film. Each donor and recipient pair is genotyped at several loci before the transplant, and informative microsatellite markers are used in subsequent studies. We are currently using CA repeat markers for mutation studies on chromosome 16q and have adopted the same polymerase chain reaction assay conditions for microchimerism analysis.

By using the technique of microsatellite sequences, Norris *et al*³¹ showed that microchimerism was present in some, but not all, posttransplant recipients. Donor type DNA was found in the peripheral blood, but none was detected from biopsies obtained from the skin and duodenum.

ANALYSIS OF THE HLA-DRB REGION

Polymerase chain reaction analysis of the HLA-DRB1 region using sequence-specific oligonucleotide probes (SSOP) or sequence-specific primers (SSP) has also been used to detect microchimerism. In SSOP, the region is amplified using generic primers. The polymerase chain reaction products are dot-blotted onto nylon membranes, hybridised with alkaline-phosphatase-labeled SSOPs, and the signal detected by chemiluminescence.³² This technique is currently being used in our laboratory for the determination of genotypes at the DRB1 locus. The sensitivity of the technique was determined by mixing experiments where the percentage of donor cells was varied against a fixed population of recipient cells. DNA from these mixed populations was then extracted and genotyped. In SSP, the primers are designed to amplify specific alleles or groups of alleles.

Investigators from King's College, London, recently reported their results for the detection of donor-specific chimerism in long-term survivors of liver transplants. The recipient and donor HLA-DRB1 region was genotyped by restriction fragment length polymorphism and by SSOP and SSP. They detected donor-specific DRB1 alleles in 39% patients using nested SSP, while SSOP and one-step SSP could detect only donor-specific chimerism in 22% and 11% of the patients tested, respectively. The donor-specific cells were more commonly seen in the skin than in the recipient bone marrow or the blood. They concluded that SSP was the most reliable technique for the detection of donor-specific microchimerism.³³

THE USE OF MONOCLONAL ANTIBODY

These are directed to the products of the donor MHC class I or II to stain tissue sections obtained from the transplant recipient, either at biopsy to rule out rejection, or at post-mortem examination. Investigators from the University of Pittsburgh¹⁴ transplanted liver and heart grafts from hamsters to rats using FK506 (Prograf, tacrolimus) as the immunosuppressive agent. Tissues from graft biopsies were stained with a standard avidin-biotin complex immunoperoxidase technique using specific monoclonal antibodies that recognise class II antigens. From 30 day onwards they demonstrated donor-type dendritic cells in the transplant recipients. Animals that showed higher degrees of cell repopulation had greater survival than those with less repopulation. Similar findings have been noted by other investigators.³⁴

QUANTITATIVE ANALYSIS OF MICROCHIMERISM

The majority of assays for chimerism are qualitative, designed primarily to establish the presence or absence of donor cells. A significant advance would be quantitation of donor cells. Two of the many approaches that may be used to achieve this goal include co-amplification (where an unrelated sequence is used as an internal reference template) and competitive polymerase chain reaction (where two similar templates share the same primer sequences). The products are analysed by densitometry following electrophoresis and autoradiography, and the level of microchimerism is obtained from a standard curve.

Conclusion

The concept of donor cell migration into recipient tissues followed by repopulation in the recipient, leading to the induction of tolerance was first put forward by Billingham *et al.*³⁵ This concept has been revived by Starzl in recent years, but it needs to be verified systematically in larger groups of transplant recipients. Recent experimental evidence has suggested that hematopoietic stem-cells residing in the donor liver were responsible for mixed chimerism after liver transplantation.³⁶ This is an area of controversy, however, as others have shown that the dendritic cell of donor leukocyte lineage was the basis of microchimerism and tolerance in recipients of solid organ transplants.³⁷ The concept of microchimerism has opened new venues of research, which may lead us to the goal of immunosuppression-free transplantation.³⁸

We thank the Showalter Trust Fund, University Surgical Associates, Fugisawa, Inc, USA, and the Patel Foundation for supporting our research.

Learning points

- microchimerism is defined as the presence of cells of donor origin in the transplant recipient
- current research is focussed on establishing the relationship between donor-specific microchimerism and clinical tolerance
- if the hypothesis that microchimerism equates with tolerance is valid, then further research to enhance the circulation of cells of donor origin in the recipient will be necessary
- one of the techniques that has been used to enhance microchimerism is to infuse donor bone marrow into the recipient, but such trials to date have been equivocal

Box 3

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