REVIEWS

Living Related Small Bowel Transplantation . . . . 526
Luca Cicalese, Pierpaolo Sileri, Cristiana Rastellini, Heran Abcarian, and Enrico Benedetti

Islet of Langerhans Autotransplantation: Rationale, Results, and New Developments . . . . . . 535
Thierry Berney, Aileen Caulfield, Jose Oberholzer, Leo Buehler, Christian Toso, and Philippe Morel

Pharmacoeconomic and Outcomes Analyses in Solid Organ Transplantation . . . . . . 544
Kathleen D. Lake

“Engineering” Myoblast Transplantation . . . . . . 558
Daniel Skuk and Jacques P. Tremblay

MEETING REPORT

TOLERANCE: DEFINING, ACHIEVING AND MEASURING IT

Report from “The Tolerance Assay: Where are we now?” Workshop, Transplant 2001 . . . . . . . . 571
Anne M. VanBuskirk and Peter S. Heeger

MISMATCHES

The Unkindest Cut: Where Are All the Transplant Programs Going? . . . . . . . . 574
Roger W. Evans

Index . . . . . . . . . . . . . . . . . . . 577
Scope

_Graft_ publishes reviews in all areas of organ and cell transplantation. These include basic immunologic topics relevant to clinical transplantation, such as tolerance induction, immunoprotection, and gene therapeutic modulation of the immune response. Other topics include xenotransplantation, tissue typing, patient selection, and operative techniques in clinical transplantation, short- and long-term graft follow-up, pharmacotherapeutic modulation of the immune response, and the physiology of grafted organs and cells.

Articles and Reviews

Reviews will be brief (2000 to 4000 words). These will generally be invited, but unsolicited proposals for reviews will be considered. We encourage color illustrations. Assistance in creating artwork can be provided by Landes Bioscience on a limited basis if necessary. Meeting reports will be invited. They are to be 1000 to 2000 words.

Other feature articles (including special forums, commentaries, and columns on ethics, technology, and managed care) should be 1000 to 2000 words in length.

**Editorial Guidelines**

**Submission**

Two printed copies of the article, in English, should be submitted. Text should be double-spaced, with page numbers throughout. Figures and disk as described below must be included. Please supply telephone and fax numbers, and email addresses if available.

Send to:

Landes Bioscience
810 South Church Street
Georgetown, TX USA 78626

**Language and Nomenclature**

Abbreviations and acronyms should be defined the first time they are used, and a list of all abbreviations should be provided. American spellings are preferred.

**Organization**

The title page must indicate corresponding author and include complete addresses for all authors, as well as an abstract. The abstract should be a maximum of 150 words. Please provide one key term definition used within the text per page of submitted article.

The introduction should describe the background of the topic. Acknowledgments should be kept to a minimum.

**References**

References for review articles are limited to 30. Important references should be annotated. References in the text are numbered consecutively as superscripts beginning with number 1. When referring to a specific reference as part of a sentence, cite as:

Example:

For a review see refs. 20 to 25.

not ...For a review see 20 to 25

The list of references should be numbered consecutively according to the order in which they are mentioned within the article. Our preferred style for reference listings is “Vancouver.” Abbreviate journal names according to the style used in Index Medicus. Spell out foreign or less commonly known journal names.

Journals: [Author's last name] [Author's initials], [Other authors' last names followed by their initials]. [Title of article with only first word capitalized]. [Journal's standard abbreviated name] [Year]; [Volume (number)]:[Pages].

Only the first 6 authors are listed. If there are more than 6 authors, the first 6 names are followed by “et al.”Initials and abbreviations are not followed by periods.

Example:


**Books**:

[Author's last name] [Author's initials]. [Other authors' last names followed by initials]. [Chapter title]. In: [Editor's last name] [Editor's initials], editor(s). [Book title]. [Number of edition]. [City]: [Publisher]; [Year]. [Pages].

Example:


Unpublished data and personal communications are not listed as references but rather appear in parentheses in the text.

**Production Guidelines**

We are an entirely Mac-based office. However, most IBM-compatible or Macintosh word processing programs are acceptable.

How to prepare text files

Our preferred word processing program is Microsoft Word; please save as version 6.0 (please no “Fast-Save” format).

Article text files should be submitted on a 3.5 inch, high-density computer disk. Save tables and figures in a document separate from text.

Figure captions, however, can be at the end of the review as text. There is no need to make a unique file for captions.

Tables will be reformatted during production, and they need only be minimally formatted in your text file. Include printouts of tables with the manuscript.

How to prepare figures, illustrations, and photos

When art is provided on disk, a single hard copy should be included to verify the illustration. It is not desirable to embed graphics within your text documents.

Compatible computer graphics programs are Adobe Illustrator, Freehand, QuarkXpress, Pagemaker, and Photoshop.

Figures and illustrations may be provided by authors as hard copy as well. Hard copies should be high-quality prints, with 2 copies of each illustration submitted. Figures will be reformatted by a graphic designer, not a medical scientist, so an enclosed figure description for complex illustrations will be appreciated and will result in improved quality.

Send only original artwork, no photocopies. Photography will be published only if the quality is reproducible. Please submit high-quality prints or slides for best quality.

All artwork should be labeled with the author's name, the figure number, and the correct orientation of the figure, but be sure that labeling is clear of the image. Do not put the label directly behind the image. Do not write directly on the back of the photograph or on the label after it has been applied. Indicate any special cropping on a photocopy of the figure.

When illustrations are reproduced from other sources, acknowledge the copyright holder at the end of the figure legend or as a footnote to tables. Do not use superscripted reference numbers in lieu of a full credit line.

**Policies**

Publication in _Graft_ implies that authors of the paper have read and agreed to its content, and that readily replaceable material described in the paper will be freely distributed to academic colleagues. Atomic coordinates, nucleic acid sequences, and protein sequences must be deposited in an appropriate data bank; papers should state that this has been done, and where possible give the entry name or accession number.

**Peer Reviews**

Each contribution to _Graft_ is rigorously vetted by at least 2 expert reviewers who are either members of the Editorial Board or are recruited by Board members. Contributors may be requested to make additions and/or changes to papers. Compliance with reviewers' recommendations is evaluated before a paper is accepted for publication.

---

**GUIDELINES**

**Language and Nomenclature**

Abbreviations and acronyms should be defined the first time they are used, and a list of all abbreviations should be provided. American spellings are preferred.

**Organization**

The title page must indicate corresponding author and include complete addresses for all authors, as well as an abstract. The abstract should be a maximum of 150 words. Please provide one key term definition used within the text per page of submitted article.

**References**

References for review articles are limited to 30. Important references should be annotated. References in the text are numbered consecutively as superscripts beginning with number 1. When referring to a specific reference as part of a sentence, cite as:

Example:

For a review see refs. 20 to 25.

not ...For a review see 20 to 25

The list of references should be numbered consecutively according to the order in which they are mentioned within the article. Our preferred style for reference listings is “Vancouver.” Abbreviate journal names according to the style used in Index Medicus. Spell out foreign or less commonly known journal names.

Journals: [Author's last name] [Author's initials], [Other authors' last names followed by their initials]. [Title of article with only first word capitalized]. [Journal's standard abbreviated name] [Year]; [Volume (number)]:[Pages].

Only the first 6 authors are listed. If there are more than 6 authors, the first 6 names are followed by “et al.” Initials and abbreviations are not followed by periods.

Example:


**Books**:

[Author's last name] [Author's initials], [Other authors' last names followed by initials]. [Chapter title]. In: [Editor's last name] [Editor's initials], editor(s). [Book title]. [Number of edition]. [City]: [Publisher]; [Year]. [Pages].

Example:


Unpublished data and personal communications are not listed as references but rather appear in parentheses in the text.

**Production Guidelines**

We are an entirely Mac-based office. However, most IBM-compatible or Macintosh word processing programs are acceptable.

How to prepare text files

Our preferred word processing program is Microsoft Word; please save as version 6.0 (please no “Fast-Save” format).

Article text files should be submitted on a 3.5 inch, high-density computer disk. Save tables and figures in a document separate from text.

Figure captions, however, can be at the end of the review as text. There is no need to make a unique file for captions.

Tables will be reformatted during production, and they need only be minimally formatted in your text file. Include printouts of tables with the manuscript.

How to prepare figures, illustrations, and photos

When art is provided on disk, a single hard copy should be included to verify the illustration. It is not desirable to embed graphics within your text documents.

Compatible computer graphics programs are Adobe Illustrator, Freehand, QuarkXpress, Pagemaker, and Photoshop.

Figures and illustrations may be provided by authors as hard copy as well. Hard copies should be high-quality prints, with 2 copies of each illustration submitted. Figures will be reformatted by a graphic designer, not a medical scientist, so an enclosed figure description for complex illustrations will be appreciated and will result in improved quality.

Send only original artwork, no photocopies. Photography will be published only if the quality is reproducible. Please submit high-quality prints or slides for best quality.

All artwork should be labeled with the author's name, the figure number, and the correct orientation of the figure, but be sure that labeling is clear of the image. Do not put the label directly behind the image. Do not write directly on the back of the photograph or on the label after it has been applied. Indicate any special cropping on a photocopy of the figure.

When illustrations are reproduced from other sources, acknowledge the copyright holder at the end of the figure legend or as a footnote to tables. Do not use superscripted reference numbers in lieu of a full credit line.

**Policies**

Publication in _Graft_ implies that authors of the paper have read and agreed to its content, and that readily replaceable material described in the paper will be freely distributed to academic colleagues. Atomic coordinates, nucleic acid sequences, and protein sequences must be deposited in an appropriate data bank; papers should state that this has been done, and where possible give the entry name or accession number.

**Peer Reviews**

Each contribution to _Graft_ is rigorously vetted by at least 2 expert reviewers who are either members of the Editorial Board or are recruited by Board members. Contributors may be requested to make additions and/or changes to papers. Compliance with reviewers' recommendations is evaluated before a paper is accepted for publication.
Living Related Small Bowel Transplantation

Luca Cicalese, Pierpaolo Sileri, Cristiana Rastellini, Herand Abcarian, and Enrico Benedetti

Intestinal transplantation recently became a valid therapeutic option for patients with irreversible intestinal failure. The vast majority of the intestinal transplants have been performed using whole intestinal grafts obtained from cadaveric donors, and fewer than 10% have been performed using segmental grafts obtained from living related donors. Intestinal living donation offers several advantages, such as minimal preservation injury, eliminating waiting time, optimal donor quality and better HLA matching and possibly reduced incidence of rejection, lower immunosuppression and side effects, possibility to decontaminate the graft prior to transplantation, and possibly reduced risk of infectious complications. In the last few years, a standardized technique has been proposed for living related small bowel transplantation (LR-SBTx). Utilizing such a technique, the authors performed a series of LR-SBTx in their center and evaluated these potential advantages. In this review, the authors summarize the worldwide experience with LR-SBTx, including their own.

**Background**

Regardless of the etiology, irreversible intestinal failure (IF) is the condition in which absorption of fluids and nutrients from the small bowel is not adequate to sustain life. Although long-term total parenteral nutrition (TPN) is adequate to support patients with IF, it is associated with important complications such as line sepsis, venous thrombosis, and hepatic dysfunction and cirrhosis. These complications are responsible for a significant mortality rate. In a recent study, patient survival on long-term TPN for nonmalignant IF has been shown to be as low as 49% at 5 years. Furthermore, the quality of life of patients on TPN is suboptimal since they often do not tolerate oral diet and are limited in their activity during the infusions. Additionally, TPN is associated with high costs. In 1992 in the United States, the estimated cost per patient per year was approximately $100,000 for supplies only, not including home nursing, physician fees, laboratory costs, and expenses related to the treatment of TPN-related complications.

Small bowel transplantation (SBTx) represents the physiologic alternative to TPN. Recent advances in immunosuppression, surgical technique, and postoperative management made SBTx a valid therapeutic option for patients with IF—with a 5-year intestinal graft survival up to 70%.

From a report of the International Intestinal Transplant Registry, approximately 300 intestinal transplants have been performed worldwide since 1985. However, the widespread application of this procedure is still limited by the relatively high rate of complications. Infections, surgical complications, acute rejection, graft versus host disease (GVHD), and posttransplant lympho-proliferative disorder (PTLD) are all observed following SBTx, with higher incidence when compared with the transplant of other organs.

The vast majority of the intestinal transplants has been performed using whole intestinal grafts (alone or in association with liver or pancreas) obtained from cadaveric donors, with or without the inclusion of the colon. However, fewer than 10% have been performed using segmental grafts obtained from living related (LR) donors.

Similarly to the transplant of other organs, intestinal living donation offers several advantages, such
as reduced preservation injury, better HLA matching, and optimal donor and graft conditions. However, this procedure cannot be performed from living donors using the standardized techniques used with cadaver grafts, and a series of transplants using LR donors has not been available to unequivocally demonstrate such advantages. Moreover, LR-SBTx has not encountered initial preference among the intestinal transplant surgeons since bowel grafts are widely available from cadavers.

In the last few years, a standardized technique has been proposed for LR-SBTx. Utilizing such a technique, we performed in our center a series of LR-SBTx and we evaluated these hypothetical advantages. In this review, we summarize the worldwide experience with LR-SBTx.

**Worldwide Experience with LR-SBTx**

The reported data on worldwide experience with LR-SBTx are summarized in Table 1. Initial attempts were reported in the 1960s and 1970s from Boston, Mississippi, and New York. In Boston, a pediatric recipient was transplanted using a segment of ileum donated from the mother and died 12 h after the procedure. From the same group, a second attempt was mentioned during the discussion of a scientific meeting, but neither of these cases was ever published.

In Mississippi, 100 cm of distal ileum was transplanted in a pediatric recipient. The graft was removed 9 days later for extensive necrosis, and the patient died shortly thereafter.

The group in New York transplanted 170 cm of jejunum and ileum between HLA identical sisters. The recipient survived 79 days, and she was able to tolerate oral diet for approximately 6 weeks. The immunosuppression used has not been reported by all these centers with the exception of New York and Mississippi where azathioprine, prednisone, and ALG were used. Although technically feasible and promising, this procedure remained a unique challenge mostly because the immunosuppression available at the time was inappropriate. The introduction of TPN in 1968 further reduced the interest in clinical SBTx. The intestine was considered the “untouchable” organ for transplant surgeons for approximately 20 years, while other solid organs were transplanted worldwide with enormous interest and impressive results in terms of graft and patient survival.

The introduction of cyclosporine elicited a new burst of interest for this procedure in the 1980s. A German group led by Deltz was the first to report a successful clinical LR-SBTx in 1988. They used a 60-cm segment of distal jejunum and proximal ileum donated by the half sister of the recipient who survived 4 years on oral diet. A previous unsuccessful attempt was performed 10 months earlier by the same group in a pediatric recipient. The 60-70 cm jejunum/ileum graft, obtained from the mother, was unfortunately rejected 12 days after the procedure. The immunosuppressive regimens used in these cases were based on cyclosporine, steroids, and ATG.

In the 1990s, a new impulse for SBTx was given by the introduction of FK-506, and LR-SBTx were performed in 5 centers. Pollard in the United Kingdom successfully transplanted a segment of 180 cm of ileum from the mother to the daughter. This patient had several episodes of rejection and died 18 months later from pneumonia. Morris, in California, reported the transplant of a segment of 110 cm of distal ileum, ileocecal valve, and cecum between twin brothers. Survival has been reported up to 1 year. The group in New Orleans, lead by Jaffe, performed 2 transplants between mother and offspring using 200 cm of jejunum. These patients had rejection and infectious complications. Survival up to 1 year has been reported. In Minneapolis, Gruessner performed 2 successful LR-SBTx from parent to offspring using approximately 200 cm of distal ileum. The author was the first to describe in detail the donor work-up and the surgical technique used to establish a standardized approach for LR-SBTx. The Japanese group of Fujimoto and Tanaka performed 2 pediatric transplants between mother and offspring using 100 to 120 cm of terminal ileum. Both patients had several episodes of rejection. One of them died 16 months after the transplant owing to Pneumocystis carinii pneumonia, whereas the other was reported alive at a 14-month follow-up.

In 1998, the first successful transplant was performed in our institution. In the following years, we performed a total of 4 adult LR-SBTx (Table 2). In our experience, the graft used was always 180 to
<table>
<thead>
<tr>
<th>Year/Place/Author/Ref.</th>
<th>Recipient Age (Yrs.)/Sex</th>
<th>Cause of IF</th>
<th>Donor HLA Match</th>
<th>Utilized Graft (Cold Ischemia Time)</th>
<th>Immunosuppression</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964 Boston (USA)</td>
<td>10, Child</td>
<td>?</td>
<td>Mother</td>
<td>ileum</td>
<td>?</td>
<td>Death 12 h after Tx</td>
</tr>
<tr>
<td>1969 Jackson at Mississippi (USA)</td>
<td>8/male</td>
<td>illeal strangulation</td>
<td>Mother, Class B (Terasaki Scale)</td>
<td>100 cm distal ileum (75 min)</td>
<td>AZA, Antilymphocyte globulin, Prednisone</td>
<td>Graft removed at POD 9 for extensive ischemic necrosis, Sepsis and death on POD 30</td>
</tr>
<tr>
<td>1972 New York (USA)</td>
<td>37/female, Gardner's syndrome</td>
<td>60 cm from the medium jejunum (80 min)</td>
<td>ATG, CsA, Steroids</td>
<td>Acute rejection after graft loss 12 days after Tx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1978 Kiel (Germany)</td>
<td>42/female, SMV and IMV thrombosis</td>
<td>60 cm lower part jejunum and upper ileum (75 min)</td>
<td>ATG, CsA, Steroids</td>
<td>4 acute rejection episodes, TPN free for 4 years when graft loss due to acute and chronic rejection, Died 5 yrs after Tx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995 Stanford California (USA)</td>
<td>34/male</td>
<td>Desmoid tumor</td>
<td>Twin brother, Identical</td>
<td>Distal ileum, ileocecal valve and portion of the cecum (110 min)</td>
<td>None</td>
<td>Sepsis-like syndrome on POD 4, Alive and TPN free at 1-year follow-up</td>
</tr>
<tr>
<td>1995 New Orleans Louisiana (USA)</td>
<td>25/female</td>
<td>Gardner's syndrome</td>
<td>Mother, Haploidentical</td>
<td>200 cm proximal jejunum</td>
<td>OLT3, FK506, MMF, Prednisone</td>
<td>Loss of 20 cm of graft on POD 7 (ischemic necrosis), Severe acute rejection 7 months after Tx, Need of night TPN after 6 months</td>
</tr>
<tr>
<td>1996 New Orleans Louisiana (USA)</td>
<td>29/male</td>
<td>Ganglioneuropathy</td>
<td>Mother, Haploidentical</td>
<td>180 cm jejunum</td>
<td>OLT3, FK506, MMF, Prednisone</td>
<td>Jejunocolostomy leakage on POD 18, 2 episodes of rejection 3 months after Tx, 4 episodes of bacterial overgrowth, 2 episodes of CMV infection, 1 candida sepsis from invasive fungal duodentitis, Need of TPN 7 months after Tx</td>
</tr>
<tr>
<td>Year, Location</td>
<td>Age/Gender</td>
<td>Family</td>
<td>Length of Ileum</td>
<td>Initial Disease</td>
<td>Transplant Type</td>
<td>Initial Therapy</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>--------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>1996 Kyoto (Japan)</td>
<td>2.5/male</td>
<td>Mother</td>
<td>100 cm distal ileum</td>
<td>Volvulus</td>
<td>FK506</td>
<td>Steroids, AZA</td>
</tr>
<tr>
<td>1997 Minneapolis (USA)</td>
<td>17/male</td>
<td>Father</td>
<td>200 cm distal ileum</td>
<td>SMA injury</td>
<td>OKT3</td>
<td>FK506, MMF, Prednisone</td>
</tr>
<tr>
<td>1997 Minneapolis (USA)</td>
<td>?</td>
<td>Mother</td>
<td>200 cm distal ileum</td>
<td>Chron</td>
<td>OKT3</td>
<td>FK506, MMF, Prednisone</td>
</tr>
<tr>
<td>1997 Cambridge (UK)</td>
<td>40/male</td>
<td>Twin brother</td>
<td>150 cm distal ileum</td>
<td>SMV thrombosis</td>
<td>OKT3</td>
<td>FK506, MMF, Prednisone</td>
</tr>
<tr>
<td>1999 Kyoto (Japan)</td>
<td>4.5/female</td>
<td>Mother</td>
<td>120 cm distal ileum</td>
<td>Midgut volvulus</td>
<td>OKT3</td>
<td>FK506, Steroids, Cyclophosphamide</td>
</tr>
<tr>
<td>1999 Geneva (Switzerland)</td>
<td>13/male</td>
<td>Twin brother</td>
<td>160 cm midileum</td>
<td>Midgut volvulus</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>1999 Xi'an</td>
<td>18/male</td>
<td>Father</td>
<td>150 cm distal ileum</td>
<td></td>
<td>PK506</td>
<td>MMF, Prednisone</td>
</tr>
</tbody>
</table>
REVIEWS

200 cm of distal ileum, donated by a family member (brother, sister, father, and mother) with excellent HLA matching (3 to 6 antigens). Three of these patients are currently alive, TPN free, and back on regular daily activities with a follow-up of 6, 21, and 36 months. No episodes of rejection or severe infectious complications have been observed. Only 1 patient developed CMV enteritis and was treated with IV ganciclovir. In the 4th patient, we had to remove the graft 6 weeks after the transplant following ischemia, probably due to octreotide treatment for severe pancreatitis. The graft had patent blood vessels and did not present immunologic or infectious complications. The patient returned to TPN and died 1 year later for TPN-induced liver failure.

Three additional successful cases have been reported worldwide in the last few years. The Cambridge group performed 1 transplant between 2 identical triplets, using a segment of 150 cm of distal ileum and no immunosuppression. Morel’s Swiss group performed a transplant between monozygotic twins using 160 cm of mid ileum. Also, a Chinese group, headed by Wang, performed an LR-SBTx between father and son using a segment of distal ileum.

Surgical Technique and Considerations

As mentioned above, cadaveric intestinal transplantation is performed using the whole intestine, whereas the LR intestinal transplant implies the use of a portion of the small bowel. It is possible to utilize segmental jejunal or ileal grafts, and both techniques have been used. However, the vascular supply of the terminal ileum offers a convenient pedicle for the graft, and this technique has been standardized. In addition, the distal ileum allows the absorption of vitamin B12, bile salts, and unlike the jejunum, a better absorption of water and solutes and is known to ensure adequate morphologic adaptation.

The approach used in our experience for LR-SBTx implies a careful donor selection. These should be young, healthy individuals for whom preoperative angiogram of the superior mesenteric artery excludes abnormalities of the vascular supply to the cecum, ileocecal valve, and terminal ileum. Furthermore, an optimal HLA matching between donor and recipient is recommended and donors should be selected, if possible, among multiple candidates accordingly. The preoperative graft decontamination is obtained with standard mechanical bowel preparation and antibiotics. A segment of 180 to 200 cm of ileum is resected 15 cm from the ileocecal valve that is spared in the donor to reduce the risk of diarrhea and liposoluble vitamin absorption impairment. In our experience, the length of the graft obtained is decided in relationship to the total length of the donor small bowel. The vascular pedicle of the graft is obtained dissecting the ileocolic vessels immediately distal to the origin of the right colic artery that is carefully preserved to maintain vascular flow to the right colon. The mesenteric peritoneum is scored, and the vessels are identified and dissected up to the origin of the ileocolic vessels. Once the segment of ileum is removed, the
remaining intestinal segments are primarily re-anastomosed in end-to-end fashion using 4-0 polyglyconate for the mucosal layer and 4-0 polypropylene for the seromuscular layer. Following vascular flush with chilled University of Wisconsin solution, the segmental graft is transplanted suturing the ileocolic vessels in an end-to-side fashion to the infrarenal aorta and inferior vena cava of the recipient using 6-0 polypropylene. Using this technique, the cold ischemia time is approximately less than 10 min and the warm ischemia time is 30-40 min. The intestinal continuity is immediately reestablished anastomosing the graft to the recipients' intestinal stumps using 4-0 polyglyconate for the mucosal layer and 4-0 polypropylene for the seromuscular layer. A temporary distal loop ileostomy is performed to monitor graft output and to perform endoscopic biopsies to evaluate rejection or viral infections. Perioperative recipient prophylaxis for infectious complications is accomplished with vancomycin (1 g IV at induction of anesthesia), piperacillin (3 g IV 6-8 times a day, adjusted for renal function, for 3 days), and ganciclovir (5 mg/kg IV every 12 h for 14 days) followed by acyclovir (800 mg PO 4 times a day for 3 months).

Our immunosuppressive protocol consists of oral tacrolimus and prednisone. Intravenous induction with atgam is used until therapeutic blood levels of tacrolimus are achieved.

Discussion

LR-SBTx offers several advantages compared with cadaveric SBTx. This is an elective procedure and can be performed when the donor and recipient conditions are optimal and donor bowel decontamination can be easily performed. This should result in a decreased risk of early infectious complications. In a previous study on recipients of cadaveric grafts, we showed that the length of preservation was a significant factor in inducing perioperative bacterial translocation (BT). With cadaveric intestinal transplant, such risk cannot be avoided since hemodynamic instability of the donor and subsequent splancnic hypoperfusion can trigger ischemic damage even before the intestine is procured. Furthermore, bowel decontamination in the donor is not feasible and these grafts are often subject to prolonged cold preservation while specific preservation solutions designed for intestinal grafts are not yet available. In a recent study, we also showed that ischemic injury induces chronic morphologic alterations of the intestinal mucosa. An additional advantage of LR-SBTx is that the availability of a living related donor allows minimization of transplant waiting time, thus reducing the evolution of TPN-related complications, such as liver damage.

An immunologic advantage is also obtained with LR-SBTx, since optimal HLA tissue matching can be obtained between donor and recipient that are related. It is a common belief that HLA matching is not important in SBTx, and this is possibly consequent to the frequent association of bowel-liver transplantation. However, no data are available from cadaveric SBTx to confirm such a belief—and a high rate of rejection, approximately 90%, have been reported in these patients. In our opinion, liver and intestinal grafts behave differently from an immunologic standpoint. In our experience with well-matched donor-recipient combinations, we have not seen rejection using an immunosuppressive regimen based on tacrolimus and prednisone. Furthermore, other groups reported LR-SBTx successfully performed between twins with low or no immunosuppression. This seems to confirm the importance of tissue matching in intestinal transplantation and, thus, should also be obtained in cadaveric SBTx since intestinal graft donors are widely available. From this experience, we adopted the strategy in our cadaveric intestinal transplant program to minimize the preservation time and to use well-matched, hemodynamically stable donors. This strategy allows a reduction of the immunosuppression, with the consequent benefit of fewer related complications. This is of particular importance since cadaveric SBTx is reportedly burdened by a high rate of PTLD up to 20%, which is higher than observed in any other organ transplant. Although unlikely in cadaveric SBTx, no cases of PTLD have been reported in LR-SBTx recipients. An additional advantage of segmental grafts is that their smaller size allows them to be transplanted in patients with a retracted abdominal cavity. This can be due to multiple laparotomies, loss of abdominal wall, or severe intra-abdominal adhesions. A potential disadvantage of LR-SBTx is the surgical risk for the donor. However, this is low if associated
with elective small bowel resection and primary anastomoses in otherwise healthy individuals, especially when the procedure is performed by experienced surgeons. To date, no surgical complications or deaths have been reported for LR intestinal donors. Furthermore, according to the available literature, it does not appear that the donor will suffer long-term absorption problems with ileal resection limited to approximately 200 cm.\textsuperscript{9,17-21} Mild occasional diarrhea can be observed only in the early postoperative time and is well controlled with medical therapy, with no evidence of vitamin B\textsubscript{12} absorption deficit or weight loss, in our experience. Additionally, to our knowledge, no long-term impairment of intestinal absorption in bowel donors has ever been reported.

An additional disadvantage of using intestinal grafts obtained from living donors rather than cadavers is the technical difficulty in using smaller diameter vessels for the vascular anastomoses. This is particularly true if the segment used is jejunum. As reported in the literature, the use of jejunum often requires multiple vessels as vascular pedicle, making the operation more challenging and increasing the risk of thrombosis or chronic hypoperfusion of the graft.\textsuperscript{19} In our experience, we utilized a single ileocolic artery and vein, performing the arterial anastomosis with interrupted technique to minimize such risks and did not witness any of these complications.

It can be argued that the use of a shorter segment of bowel in LR-SBTx may not be sufficient to provide an adequate absorption of nutrients. From the literature, most of the surgeons performing LR-SBTx have used segmental grafts of 160 to 200 cm. The decision on how to select an optimal length of bowel is purely empiric. However, it is based on the knowledge that a segment of 50 cm of small intestine will not allow sustaining of life with enteral alimentation.\textsuperscript{33-35} Considering that the graft can undergo injury for manipulation, preservation, and rejection, we believe that it is safe to use a segment of 180 to 200 cm of ileum. The choice of this length also ensures that the donor is left with a segment of at least 300 cm of native small bowel and terminal ileum that are not subject to similar damages. Furthermore, the preservation of the ileocecal valve in the donor contributes to reducing postsection dehydration. In our experience, the segmental grafts underwent complete functional adaptation within 6 months. These patients were TPN free immediately after the transplant and able to regain—and maintain—preintestinal failure body weight and serum albumin levels with oral diet.\textsuperscript{36}

After cadaveric SBTx, bacterial, fungal, and viral infections are quite common. The incidence of such complications is higher than any other organ transplant, probably due to the need for more vigorous immunosuppression. Infectious complications are the most common cause of death and graft loss, accounting for up to 69% of patient loss after cadaveric SBTx.\textsuperscript{9} Line infections, sepsis, abdominal fungal infections, and viral infection or re-infections (EBV and CMV) are also reported after LR intestinal transplantation. Although less frequent than cadaveric SBTx, severe infections leading to recipient death have been reported.\textsuperscript{19,20} Several authors speculate that some of these infectious complications originate from bacterial translocation of enteric flora during rejection episodes.\textsuperscript{17} Recently, we analyzed the number of bacterial translocation episodes (evaluated by the simultaneous presence of a specific microorganism in the stool and other sites) in 50 pediatric SBTx recipients.\textsuperscript{26} This analysis showed that 44% of patients had at least one episode of BT associated with rejection and cold preservation. In a recent analysis of our LR-SBTx experience, we observed a very low rate of infections and no episodes of BT.\textsuperscript{38} It is difficult to extrapolate any conclusion since our experience is limited, but the absence of bacterial infections and the low rate of viral complications observed suggest an advantage to this approach. Several factors might have contributed in this regard, such as hemodynamic stability of donors and recipients, optimal graft decontamination, minimization of preservation injury, and reduced immunosuppression. However, it is impossible to identify which of these factors plays a dominant role and probably they all contribute in part to reducing BT and infectious complications in LR-SBTx.

Despite all these considerations, several attempts performed worldwide with LR-SBTx have been unsuccessful. However, long-term patient and graft survival were achieved with LR-SBTx, even in the pre-tacrolimus era in some patients, probably due to some degree of immunologic advantage obtained.
with the tissue matching. Another limitation of the reported experience with LR-SBTx is the dishomogeneity of the cases. Often, these were performed as isolated attempts by each group, making it impossible for the surgeon to overcome an unavoidable learning curve. Furthermore, different surgical techniques were often used as well as different immunosuppressive regimens (Table 1). In our experience, we used a standardized approach to evaluate the potential advantages of LR compared with cadaveric SBTx.

In conclusion, intestinal living donation offers several advantages, such as minimized preservation injury, eliminating waiting time, optimal donor quality, and better HLA matching and reduced incidence of rejection, lower immunosuppression and reduced associated side effects, possibility to decontaminate the graft prior to transplantation, and reduced risk of infectious complications. From the reported cumulative experience, LR-SBTx reached a 1-year survival rate of approximately 50%. Evaluating the reported cases in the tacrolimus era, the survival rate at 1 year goes up to approximately 70%. In our opinion, these rates are not reflecting the real potential of the procedure. As we already discussed, different groups utilized many different approaches, creating confusion without gaining extensive experience. We suggest that a standardized approach should be used for LR-SBTx. In our limited but significant experience with this procedure, we are confident that LR-SBTx is a valid alternative to cadaveric SBTx.

Significant advantages offered by this approach such as short ischemia time, HLA match, and selection of hemodynamically stable donors should be, in our opinion, adopted for cadaveric intestinal transplantation as well.

References

Islet of Langerhans Autotransplantation: Rationale, Results, and New Developments

Thierry Berney, Aileen Caulfield, Jose Oberholzer, Leo Buchler, Christian Toso, and Philippe Morel

Autotransplantation of islets of Langerhans should be offered to patients undergoing extensive pancreatic resection for chronic pancreatitis. Results of clinical trials of islet autotransplantation (in which allorejection and recurrence of autoimmunity do not exist as causes of graft destruction) have been superior to those of allotransplantation, with insulin independence for more than 1 year achieved in 47% of recipients. The number of islets transplanted is a major indicator of outcome, since insulin independence at 1 year increases to 71% in recipients of more than 300,000 islets. Importantly, long-term pain control after extensive pancreatic resection is excellent and reaches 82% to 100%. Even in patients who achieve insulin independence, responses to intravenous glucose challenge are depressed and functional insulin secretory reserve is markedly decreased, indicating that only a reduced mass of islets engrafts. New indications for islet autotransplantation are emerging and include benign pancreatic tumors, blunt trauma, and, more controversially, malignant tumors of the pancreas.

Introduction

Islet of Langerhans transplantation is in the limelight, thanks to remarkable results recently obtained by the Edmonton group after islet allotransplantation in type 1 diabetes mellitus patients. A new surge of interest has been generated and is likely to benefit other domains of islet transplantation, notably autologous transplantation for the prevention of surgical diabetes. This is an interesting role reversal, since autotransplantation was recently viewed from a technical standpoint as a critical model for studying the determinants for successful islet transplantation in the absence of immunological mechanisms of graft loss, and thus as a first step to master before successful islet allotransplantation. Indeed, successful results of functional islet autotransplantation after extensive pancreatectomy were frequently obtained, as compared with the dismal outcome of a vast majority of allogeneic transplantation procedures. A number of factors doubtless account for the differences observed, including the absence of administration of diabetogenic drugs (steroids and calcineurin inhibitors), allogeneic rejection, and the recurrence of autoimmunity. Other not-as-well-defined mechanisms, such as the result of the interaction between the islet graft and the microenvironment at the site of implantation, might also be involved in islet graft loss.

Surgical diabetes, provoked by extensive pancreatic resection, is a condition comparable in severity to type 1 diabetes. Chronic pancreatitis is the most common indication for extensive pancreatic resection. Such patients are hyperglycemic and at risk of ketosis in the absence of exogenous insulin. They suffer frequent hypoglycemic episodes, resulting from a lack of counterregulatory mechanisms (i.e., absence of glucagon), and of poor compliance in the context of chronic alcohol abuse. On the other hand, extensive pancreatic resection is often required for patients with intractable pain due to chronic pancreatitis, and islet autotransplantation...
Chronic Pancreatitis: to Resect or Not to Resect?

Patients suffering from chronic pancreatitis (CP) are usually referred to the surgeon for chronic intractable abdominal pain. The type of surgical treatment is a matter of controversy, but it is generally accepted that pancreatic duct drainage should be performed in the presence of a dilated duct, whereas resection should be offered to patients with “small duct disease.” However, this principle has been challenged by the failure to obtain pain relief by pancreaticojejunostomy in a number of patients with “enlarged duct” CP. The notion that the pancreatic head might be the “pacemaker” of the disease in alcohol-induced chronic pancreatitis and the fact that damage to nerves located around and within the pancreatic inflammatory mass plays a significant role in the generation of pain are likely explanations for failed duct drainage procedures. Suspect of carcinoma or local complications, such as thrombosis, pseudoaneurysms, pseudocysts, and compression of the biliary or digestive tracts, also indicate the performance of a resection procedure.

Both distal pancreatectomy and pancreatoduodenectomy have been associated with growing safety—with mortality rates under 1%. Good quality of life and satisfactory long-term pain control are achieved by an appropriate resection procedure in about 90% of cases. A significant number of patients have a long-lasting history of pain and undergo multiple surgical procedures before pancreatic resection is decided on, suggesting that resection is often considered and performed too late in the course of disease.

In the extreme, total or near-total pancreatectomy is the most effective procedure in relieving pain, but it invariably results in insulin-dependent diabetes. Surgical diabetes is severe and difficult to manage: patients develop hyperglycemia and are at risk of ketoacidosis in the absence of insulin therapy. They may also develop long-term diabetic complications if they live long enough. Moreover, they present frequent hypoglycemic episodes because of poor compliance in a context of continued alcohol abuse, and because of a lack of the counterregulatory mechanisms provided by glucagon. Therefore, the possibility of preserving endocrine function through islet autotransplantation would be a significant asset for pancreatectomized patients.

Another important consideration when balancing the metabolic risks and symptomatic benefits of extended pancreatic resection resides in the natural history of chronic pancreatitis. A prospective series of 245 patients reported a 74% incidence of diabetes with a median time of 5.7 years from diagnosis. We have reported a 26% diabetes-free survival at 10 years after pancreatic resection for CP, with no difference regarding type (duodenopancreatectomy vs. distal pancreatectomy) or extent of resection. These findings illustrate the relentless character of the disease with an almost inexorable progression toward total glandular destruction. They might provide a rationale for the performance of earlier and more extensive pancreatic resection and islet autotransplantation in order to provide these patients, who are inexorably headed toward diabetes, with a larger number of healthier islets. Although these considerations remain controversial, islet autotransplantation should nonetheless be offered to any patient with CP undergoing extensive pancreatic resection.

Experimental Islet Autotransplantation

The door to successful clinical islet autotransplantation was opened with the description of new methods for the isolation and transplantation of islets of Langerhans in rodents, and the demonstration of diabetes reversal after the transplantation of syngeneic islets in animals with “chemical pancreatectomy” induced by streptozotocin injection. However, the experiments conducted in inbred rodents did not reflect the technical difficulties that are encountered when applying the method for application in larger mammals, including the human. Studies performed on large animals to demonstrate the feasibility of diabetes reversal by islet autotransplantation have been instrumental in applying the concept to the clinical situation. Reinfusion of islets isolated after total pancreatectomy into the portal system was shown to result in consistent long-term correction of surgical diabetes in subhuman primates (dogs and pigs) and enabled the
quantification of the critical mass of islet tissue necessary to revert diabetes in each species.\textsuperscript{23-26}

Much research was conducted in the search for an optimal implantation site for the islets. The liver (by intraportal infusion) and the spleen (by retrograde infusion into the splenic vein) were consistently identified as the most favorable sites for implantation of purified autologous islets in large mammals, as demonstrated by rate of engraftment or posttransplant metabolic studies.\textsuperscript{23-27,29} The theoretically more physiological insulin secretion, directly into the portal vein of the splenic location, does not seem to offer significant advantages. Interestingly, free intraperitoneal islet autotransplantation showed better engraftment and long-term endocrine function when unpurified dispersed pancreatic tissue was compared with purified islets in canine models.\textsuperscript{28,30} Moreover, long-term autograft function of intraperitoneal unpurified tissue was similar to that of intrahepatic purified islets.\textsuperscript{30} Omental pouches were designed in a canine model as ideal transplant sites, combining the advantages of insulin secretion into the portal flow with easy retrievability for biopsy purposes. However, this method required a significantly larger autologous islet mass to reverse diabetes than did the intrasplenic site.\textsuperscript{31} The kidney capsule is a highly favored transplantation site in rodents because of the technical simplicity of the procedure and the possibility of demonstrating graft function by observing a return to diabetes after nephrectomy. Analysis of nonimmunologic mechanisms of graft failure can be performed in murine models of transplantation of a marginal mass of syngeneic islets under the kidney capsule.\textsuperscript{32,33} However, largely because of lack of engraftment, which is likely due to poor vascularization of the graft site,\textsuperscript{23,28,34} poor functional outcome is achieved after transplantation of purified autologous islets under the kidney capsule of large mammals.

With islets isolated from healthy animals, autotransplantation falls short of the situation encountered when dealing with patients with chronic pancreatitis, in which islets must be isolated from a fibrous and scarred pancreas. In an attempt to reproduce the clinical situation, islet isolation and autotransplantation in canine models of chronic pancreatitis induced by duct ligation achieved diabetes reversal in, at best, 50\% of recipients, a result of low yields, but demonstrated the feasibility of the method.\textsuperscript{35-38} Animal models have allowed extensive studies of the metabolic function of the autotransplanted islets. Such studies pointed out that, in spite of a euglycemic status, autotransplanted animals had impaired glucose responses to glucose tolerance tests\textsuperscript{39} and markedly reduced insulin responses to glucose and arginine, the latter parameter being a direct measure of the islet secretory capacity, that is, the engrafted islet mass.\textsuperscript{29,40} The defective glucagon response to hypoglycemia, observed after human intrahepatic islet autotransplantation,\textsuperscript{41} could be reproduced in a canine model but was restored when islets were transplanted intraperitoneally.\textsuperscript{42} This finding suggested that the defective glucagon response may not solely be the result of an isolation-induced destruction of $\alpha$-cells or a lack of autonomous innervation, and was tentatively explained by the lack of a proper hypoglycemic stimulus in the hepatic site because of high glucose concentrations in the microenvironment.\textsuperscript{32,33} Interestingly, basal pancreatic polypeptide (PP) levels were consistently low, suggesting a loss of the vagally mediated PP response to hypoglycemic stimuli.\textsuperscript{39,42}

**Technical Considerations for Human Islet Autotransplantation**

When autologous islet transplantation is considered, the surgeon must preserve the vascularization of the pancreas until its final removal to minimize the ischemic injury to the gland. The pancreas is immediately transported to the isolation laboratory, and the islets are isolated with a collagenase digestion method. Liberated by enzymatic digestion, the islets are traditionally not purified from the dispersed ductal and exocrine tissue, mainly to maximize yield.\textsuperscript{44} This also reduces the processing time of the pancreatic tissue, which can be ready to infuse in less than 2 h, during which pancreatic surgery can be completed.\textsuperscript{45} Transplantation of unpurified dispersed pancreatic islet tissue was introduced by the Minneapolis group after they had shown that it could successfully reverse diabetes in pancreatectomized dogs.\textsuperscript{46,47} However, the extra volume of tissue to be transplanted, and the potential presence of activated pancreatic enzymes in the absence of purification, carries an increased


risk of portal hypertension and/or thrombosis and intravascular coagulation.46-51 For these reasons, certain groups prefer to purify the pancreatic digest on density gradients prior to transplantation.48-51 The automated method for islet isolation,49 in which the pancreas is fully immersed in a chamber with a 400 to 500 µm screen filtering the outlet where it undergoes continuous enzymatic digestion by a 37 °C collagenase solution circulating in a closed circuit, can be used effectively to separate islets from glands with CP. It also offers the advantage of a partial purification because the fibrous components of the pancreas are retained in the chamber.54

The dispersed islet tissue is brought back to the operating room for intraportal infusion. Islets are infused via a catheter inserted inside a branch of the mesenteric vein after systemic heparinization.45,51,55 Since the volume of the unpurified digest can be as high as 35 to 45 ml, the infusion is performed slowly and under constant monitoring of the portal vein pressure. Peak portal pressures, as high as 70 cmH2O (50 mmHg), have been recorded during islet infusion.55-55 The upper safety limit at which infusion should stop is not well defined and obviously depends on the pretransplantation value. The Minneapolis group has opted to inject the remaining tissue freely into the peritoneal cavity when portal vein pressure reaches 40 cmH2O (30 mmHg).45

The spleen has been explored as an alternate site for islet autotransplantation.51 It has the theoretical advantage of a more physiological location upstream from the liver and is able to sustain islet function in canine models.29 The islets are transplanted by retrograde venous infusion, generally into a short gastric vein. However, even if this solution is feasible and can lead to insulin independence, it has been associated with an increased rate of thrombotic complications, which implies that the performance of spleen preservation during pancreatic resection in an inflammatory terrain may be difficult.51

Interestingly, the lack of an in-house islet isolation facility is not an obstacle for the performance of islet autotransplantation after pancreatectomy. A group in Portland, Oregon, has reported on 5 patients, for whom resected pancreata were shipped in cold preservation solution to Minneapolis for processing and the dispersed tissue was shipped back for infusion. Islet transplantation was performed after a 16- to 24-h delay via a percutaneous mesenteric vein catheter positioned during surgery and continuously flushed with low-volume dilute heparin solution. Satisfactory long-term results in terms of insulin requirements demonstrate that distant processing of islet tissue for autotransplantation is a feasible and reasonable option.56

Results of Clinical Islet Autotransplantation

The latest newsletter of the International Islet Transplant Registry (ITR) reports 240 autologous islet transplant procedures performed through December 2000 in 15 institutions worldwide.3 Early experience in the 1970s and early 1980s, under the pioneering leadership of the Minneapolis group, demonstrated the feasibility of islet autotransplantation after near total or total pancreatectomy, with some success in preserving metabolic function.7,20,37-50 Results of these small series of selected cases are difficult to interpret, but an exhaustive analysis of the published early experience showed that, overall, 32% to 57% of patients achieved at least transient insulin independence, depending on the extent of pancreatectomy.41

Between 1990 and 1999, the ITR reports that 64% of patients were insulin independent for more than 1 week and 47% for more than 1 year. If more than 300,000 islet equivalents (IEQ: number of islets if all had an idealized diameter of 150 µm) were transplanted, this proportion rose to 71%, with a longest insulin independence follow-up of more than 13 years (Fig. 1).52 The most active centers in the past decade have been Minneapolis, MN; Leicester, UK; Geneva, Switzerland; and Indianapolis, IN.45,51,55,55 Recently published results by these institutions are summarized in Table 1 and show a marked improvement in the achievement of sustained insulin independence. In the Minneapolis series, islet yields and probability of insulin independence after islet autotransplantation were significantly increased after the introduction of the automated method for islet isolation in 1991.45 Unsurprisingly, the major determinant of success (i.e., insulin independence) for islet autotransplantation is the number of islets infused, either calculated as the number...
Table 1  |  Functional Results of Islet Autotransplantation in the 4 Most Active Institutions Between 1990 and 1999 According to the International Transplant Register

<table>
<thead>
<tr>
<th>Institution</th>
<th>Year</th>
<th>N</th>
<th>IEQ Totala</th>
<th>IEQ/kgb</th>
<th>Insulin Independence &gt;1 Month</th>
<th>Insulin Independence &gt;1 Year</th>
<th>Sustained Insulin Independenceb</th>
<th>Longest Insulin Independence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minneapolis (44,62)</td>
<td>1995</td>
<td>48</td>
<td>238,010 (400-1,076,000)</td>
<td>n/a</td>
<td>20 (51%)</td>
<td>14 (45%)</td>
<td>15 (38%)</td>
<td>13 years</td>
</tr>
<tr>
<td>Indianapolis (51)</td>
<td>1998</td>
<td>6</td>
<td>223,667 (83,000-415,000)</td>
<td>3,702 (1,630-6,290)</td>
<td>6 (100%)</td>
<td>1/1 (100%)</td>
<td>6 (100%)</td>
<td>12 months</td>
</tr>
<tr>
<td>Geneva (54)</td>
<td>2000</td>
<td>13</td>
<td>163,383 (23,904-450,000)</td>
<td>2,599 (386-6,716)</td>
<td>11 (85%)</td>
<td>7/11 (64%)</td>
<td>7 (54%)</td>
<td>4.5 years</td>
</tr>
<tr>
<td>Leicester (64)</td>
<td>2001</td>
<td>24</td>
<td>140,419n</td>
<td>2,604 (320-9,240)</td>
<td>8 (33%)</td>
<td>4 (n/a)</td>
<td>4 (17%)</td>
<td>3 years</td>
</tr>
<tr>
<td>Pooled results</td>
<td></td>
<td>91</td>
<td>n/a</td>
<td>2,940</td>
<td>45 (49%)</td>
<td>n/a</td>
<td>32 (35%)</td>
<td>13 years</td>
</tr>
</tbody>
</table>

a References are indicated in parentheses; b year of publication; c number of transplanted patients in each series; d total number of islet equivalents (IEQ) isolated and available for transplantation (mean). IEQ are number of islets normalized to a diameter of 150 µm; number of IEQ transplanted per kilogram body weight (mean); several patients have less than 1-year follow-up; number of insulin-independent patients at latest follow-up or death; only 39 patients were included for long-term analysis; total number of islets transplanted (number of IEQ not available); n/a: data not available; actuarial value; 5 patients underwent intrasplenic islet infusion; and median value (mean not available).

of islets transplanted, with an optimal number above 200,000 to 300,000 islets,41,45,64,65 or as the number of IEQ per kilogram of body weight, with an apparent cutoff value of 2500 to 3000 IEQ/kg.55,57,66

Even if insulin independence was not achieved, nearly all patients in the Geneva and Leicester experiences had functioning grafts as measured by basal C-peptide production, and HbA1c levels and 24-h insulin requirements were significantly lower than in patients who underwent total pancreatectomy without islet autotransplantation.55,66

“Burn-out” of a functional islet graft can occur after prolonged insulin independence, but patients in whom the size of the graft is sufficient to function for more than 2 years apparently do not fail beyond that point;44 although this view has been challenged by occasional observations of later graft failure.51,55

The islet yield of the isolation procedure greatly depends on the extent of fibrosis in the resected pancreas, as demonstrated by a negative correlation between number of islets recovered and the degree of pancreatic fibrosis.45 For example, at the University of Geneva the mean islet yield after isolation was 3494 IEQ per gram of resected pancreas and was significantly lower in patients with chronic pancreatitis than in patients with normal pancreatic tissue (2044 IEQ/g vs. 5184 IEQ/g).55

A history of previous pancreatic resections will also influence the islet yield, since less pancreatic tissue will be available for islet isolation with a completed pancreatectomy.

The extent of pancreatic resection does not seem to affect the rate of insulin independence achieved, that is, insulin secretion by the pancreas remnant is unlikely to play a significant role in the posttransplantation metabolic status.55,55 This is unsurprising given the poor mid- to long-term endocrine function of CP pancreata regardless of therapeutic option.18,21

Importantly, long-term pain control results have been excellent, with resolution or improvement of pain in 82% to 100% of patients,19,45,63 and far better than those achieved in CP patients who underwent duct drainage procedures or minor pancreatic resection.19

Complications

Morbidity related to pancreatic resection for CP is significant and has been reported and discussed elsewhere,15,18,68 but complications directly attributable to islet infusion are much rarer. However, it
should be remembered that the early days of islet autotransplantation were marked by reports of serious, and often fatal, complications, seemingly involving a chain of events that began with acute portal hypertension and led to disseminated intravascular coagulation (DIC), and occasionally was accompanied by portal vein thrombosis and hepatic infarction.48,49,69,70 Pancreatic enzymes, trypsin in particular, have long been known for their thrombogenic properties and ability to lead to DIC if released into the bloodstream, an effect that can be blocked by heparinization.71 In addition, commercial crude collagenase preparations were shown to activate proteolytic pancreatic enzymes during the digestion process.72 These factors may well explain the development of DIC after infusion of unpurified pancreatic digest into the portal system. Indeed, since the advent of the automated method of islet isolation, in which partial purification of the pancreatic tissue is achieved,74 and with the availability of a new generation of gentler enzyme blends75 and the routine administration of heparin,45,48 DIC has no longer been reported after infusion of autologous islets into the portal vein.

The only significant complications of islet autotransplantation recently reported have been 2 cases of partial portal vein thrombosis and 1 wedge splenic infarct (all 3 without functional consequence), 1 case of splenic vein thrombosis after intrasplenic infusion, and 2 cases of splenic hilar bleeding after intraportal infusion (all 3 leading to splenectomy).45,51,66 One case of fatal DIC also occurred after intrasplenic islet infusion, secondary to microembolization into the lungs of pancreatic tissue fragments that migrated through portosystemic collaterals.50

The invariable elevation of intraportal pressure that occurs during islet infusion may understandably lead to a marked decrease of the portal blood velocity, with ensuing thrombosis. However, there may be more to these thrombotic events than the sheer effect of a large mass of tissue carrying activated proteolytic enzymes. Interestingly, it was recently shown in allogeneic and xenogeneic in vitro models that isolated islets infused into the bloodstream could activate the coagulation and complement cascades, thus leading to clot formation and platelet consumption.74,75 This phenomenon is likely of significance in an autologous situation as well.76 Finally, for reasons that mostly remain unclear, intraportal infusion has been associated with fewer complications than intrasplenic infusion and should therefore be the preferred site for autologous islet infusion.50,51,66

**Metabolic Studies in Recipients of Autologous Islet Transplants**

Preoperative assessment of the pancreatic endocrine function should be obtained by oral and/or
intravenous glucose tolerance tests (IVGTT) and intravenous glucagon challenge because it is easy to foresee that a patient with impaired metabolic tests, let alone established diabetes, is unlikely to become euglycemic after islet autotransplantation. Indeed, in the Minneapolis series, almost all patients had normal or near-normal pretransplant glucose tolerance tests.

Normal IVGTTs, defined by a K value (glucose disposal rate) greater than 1% per minute, are often observed when insulin independence is achieved after islet autotransplantation and correlate significantly with the number of islets infused. However, K values are usually higher before isolation, although they have occasionally improved after transplant. Similarly, acute insulin responses to intravenous glucose or to arginine are consistently lower in euglycemic islet autograft recipients with normal HbA1c, as compared with healthy controls or pretransplant values.

Functional insulin secretory reserve, measured by glucose-potentiated, arginine-induced insulin secretion 3 years after pancreatectomy and autotransplantation in 8 patients with sustained insulin independence, correlated highly to the mass of islets transplanted. Despite insulin independence and normoglycemia, the response was markedly decreased in all patients when compared with matched controls, indicating that only a reduced mass of islets had engrafted. In further metabolic studies, this group of patients had no glucagon response to insulin-induced hypoglycemia, and a depressed but positive glucagon response to arginine. Similar observations were made in autografted patients after 2.5 years of insulin independence and normoglycemia during hypoglycemic hyperinsulinemic clamp studies. The fact that a glucagon response is obtained after arginine stimulation indicates that loss of α-cells is not responsible for this observation. These findings have been verified in animal models and are discussed above. The defective glucagon response was not observed in recipients of whole organ pancreatic allografts after pancreatectomy.

PP responses to insulin-induced hypoglycemia or to the high-protein meal are completely absent, whereas recipients of pancreatic allografts had a PP response only to the high-protein meal, but not to insulin. No definite explanation has been offered for this observation.

New Indications
The increasing success of islet autotransplantation after pancreatectomy for CP has prompted the Geneva group to expand the indications for the procedure. We have transplanted islets isolated from 6 pancreata resected for other benign pathologies (3 cystadenomas, 2 insulinomas, 1 blunt trauma to the pancreas). Median percentage of resected tissue was 80%. Five of these 6 patients have sustained insulin independence after a median follow-up of 35 months. Caution must be applied when transplanting islets isolated from supposedly benign tumors, and a diagnosis of malignancy must be unequivocally ruled out before making the decision to perform the transplant, especially if the decision for tumor removal arises from preoperative diagnostic uncertainty. However, this approach can be useful for benign lesions whose size and/or location (neck and body of the pancreas) require the performance of an extended pancreatic resection to achieve complete extirpation.

More controversially, total pancreatectomy, combined with islet autotransplantation, was recently proposed as an option for the treatment of pancreatic adenocarcinoma. This was reported in one patient who underwent completion of a proximal pancreatectoduodenectomy for a life-threatening anastomotic leakage, and who is alive with a functional islet graft 1 year after the procedure. Obviously, a curative pancreatic resection and the infusion of islets uncontaminated by tumoral cells are prerequisites for the performance of such a procedure. Detection of the K-ras mutation by PCR in the islet preparation might be a useful technique to prevent infusion of contaminated islets.

Conclusions
Numerous advances in understanding mechanisms of islet graft loss at the cellular and molecular levels, in the development of new reagents for islet isolation and purification, and in the clinical management of islet graft recipients, have led to significant improvement and success in the functional results of islet of Langerhans transplantation. As a result, an increasing number of centers are
launching islet transplantation programs. This is likely to lead to an increase in the number of autotransplant procedures after pancreatic resection for chronic pancreatitis or other indications. The islet transplant community will have to take advantage of this ideal situation for the implementation of multicenter, prospective randomized trials, aimed at validating the concept of pancreatic resection/islet autotransplantation. Regarding long-term metabolic results, such studies should focus on determining the optimal timing for, and extent of, pancreatic resection, as well as identifying selection criteria and providing guidelines for pancreatic resection and islet autotransplantation, in comparison to more conservative approaches.

References


Pharmacoeconomic and Outcomes Analyses in Solid Organ Transplantation

Kathleen D. Lake

A number of new immunosuppressive agents have been introduced within the past decade. Each of these agents has produced impressive results in Phase III clinical trials, with acute rejection rates declining from the 40% to 50% range to well under 15% to 25% with newer immunosuppressive combinations. However, with the addition of each agent comes an incremental increase in the cost of therapy, resulting in maintenance regimens that vary in price from $1,700 with azathioprine and prednisone to well over $16,000 per year for some of the newer, more potent combinations. Pharmacoeconomic and outcomes analyses can assist practitioners in identifying optimal strategies for patients when selecting among a number of highly effective but costly agents. Utilization of these techniques, in combination with the evidence-based medical literature, allows healthcare decision makers to make both scientifically and economically sound decisions. The intent of this article is to provide a review of the current pharmacoeconomics literature for transplantation.

Introduction

Over the past decade, progress in the field of transplantation has been accompanied by an increased emphasis on controlling the overall costs associated with it. The average billed charges for the various transplantation procedures in 1999 were $111,400 for kidney, $303,300 for heart, and $244,600 for liver (Table 1).1-2 Discounted contract reimbursement and Medicare/Medicaid reimbursement typically run much less for any given procedure. Managed care organizations have also implemented the use of contracts based on capitated or global payments inclusive of the transplant hospitalization, physician fees, certain periods of follow-up care (first 90 days to 1 year), and in some cases, also include consultant fees. These types of reimbursement strategies have placed an increased burden on transplant centers to share the risk and has forced them to evaluate both the cost and the effectiveness of various treatment regimens and procedures. Patients also feel the increased pressures of healthcare reform with higher copays, limited lifetime maximums on insurance coverage, and insurers dictating where patients may have their transplants performed (i.e., “centers of excellence”). Costly maintenance immunosuppressive regimens may “spend down” the allocated resources more quickly for a given patient, but this apparent disadvantage must be weighed against the cost of expensive complications, including the possible return to dialysis or need for retransplantation.

To complicate the financial issues further, a number of new immunosuppressive agents have been introduced during the past decade. Many of the multicenter trials have reported impressive results, with acute rejection rates declining from the 40% to 50% range, with cyclosporine and prednisone, with or without azathioprine, to well under 15% to 25% with the newer 3 or 4 drug combination cocktails.3-9 However, the addition of each agent is associated with an incremental increase in the overall cost of immunosuppressive therapy.10 Maintenance
regimens vary in price from $1,700 with azathioprine and prednisone to more than $16,000 per year for some of the more potent regimens (Table 2). When reviewing the various multicenter clinical trials, it is apparent that similar reductions in the incidence of acute rejection can be achieved with different regimens. The following question is then called for: Is it possible to achieve the same outcome at a lower cost or a better outcome at the same cost? Certainly, drug therapy for transplantation is expensive; however, this is overshadowed by the costs associated with treating the consequences of failed immunosuppressive therapy. Even though there is a wide variation in reported costs associated with major complications following solid organ transplantation, it is well recognized that the loss of a kidney graft and the return to dialysis and/or the transplant waiting list is neither cost-effective nor beneficial to the patient's quality of life.11-13

In the early days of economic analyses, a common, albeit shortsighted, approach was to look only at the actual cost of the given medications, assume outcomes were equivalent, and then use the cheapest product. If that practice were in use today, immunosuppressive regimens consisting of azathioprine and prednisone might still be the mainstay of

### Table 1

<table>
<thead>
<tr>
<th>Organ Acquision and Transplant Procedures (in dollars)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1999 Mean Local Standard Acquisition Charges by OPOs</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Acquisition</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>Kidney/Pancreas</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Heart/Lung</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Double Lung</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Small Intestine</td>
</tr>
</tbody>
</table>

*1999 APO Annual Report; **Milliman and Robertson's 1999 report.

### Table 2

<table>
<thead>
<tr>
<th>Typical Immunosuppressive Regimen Cost (Average wholesale price)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neoral Cyclosporine (microemulsion)</strong></td>
</tr>
<tr>
<td>$25 mg/kg per day</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>13,100-13,400</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>X</td>
</tr>
</tbody>
</table>
therapy. Fortunately, the focus has gradually shifted from using the acquisition cost of the agents to evaluating the overall benefits derived from the therapy. Short-term benefits of the various immunosuppressive regimens are typically measured in terms of avoidance of acute rejection and adverse effects. Ideally, economic comparisons should consider not only these short-term resource savings but also potential long-term benefits, such as improved patient and graft survival, as well as improvements in health-related quality of life. It is important to recognize that the more successful the immunosuppressive regimen is in extending both patient and graft survival, the more cost-effective it will be. Improved long-term outcomes will ultimately benefit society in several ways, including the following:

1. reducing the number of retransplant procedures, allowing the existing organ supply to be used for first-time transplants;
2. reducing the time spent on dialysis and the waiting list; and
3. improving the overall efficiency of the transplantation system.

**Types of Pharmacoeconomic Analyses**

Pharmacoeconomics is typically considered a subset of outcomes research that deals specifically with pharmaceutical interventions. The therapy can be compared with other drugs, invasive and noninvasive therapy, or even watchful waiting. Pharmacoeconomic analyses can be divided into 2 categories: economic evaluations and humanistic evaluations (Table 3). These studies can be viewed from a number of perspectives, including that of society, the payer, the patient, the provider, or the producer. Specific methods for performing these studies are reviewed elsewhere.14-16

Pharmacoeconomic studies attempt to examine total resource consumption, or all costs associated with monitoring a given therapy, including the acquisition cost of the drugs, the cost of providing follow-up services, the cost of side effects, and any other costs such as concomitant medications. Utilization of charge data is often misleading because of cost-shifting that may occur in an institution.17 Costs can be divided temporally, into those that occur either in a pretransplant environment (e.g., evaluation and managing the patient’s chronic disease), those that occur during the actual transplant itself (e.g., hospitalization-related costs), or those that occur subsequently (e.g., immunosuppressants, rejection therapy 1 year posttransplant, etc.). Clinical outcomes specific to transplant, which need to be accounted for in cost-consequence modeling, include the clinical disease features of rejection, infection, and chronic rejection. Also to be considered are adverse events such as nephrotoxicity, hypertension, hyperlipidemia, and steroid-related complications, and the need for retransplantation along with the attendant possible consequence of mortality. Some of the pertinent variables in cost-consequence modeling for transplantation are described in Table 4. Most of the existing pharmacoeconomic analyses have limited their focus to 1 or 2 of the major drivers of the transplant process (re-
Reviews

Exclusion of the procedure costs, organ procurement fees, or even initial hospitalization may be appropriate, assuming that the use of a given agent will have little impact on certain factors. Others have highlighted the importance of focusing on the immunologically relevant variables most likely to be affected by a regimen or a given procedure and would exclude those aspects unrelated to transplantation (e.g., hospitalization for a motor vehicle accident is unlikely to be related to immunosuppressive regimen but could dramatically increase length of stay or charge/readmission for a given patient).17

Humanistic Evaluations

Economic advantages have been well documented for renal transplantation as compared with dialysis and other healthcare interventions.1-13,18-20 Health-related quality-of-life (HR-QOL) benefits have been described for various types of organ transplantation.21 Shield et al. showed that patients who were receiving dialysis for end-stage renal dysfunction had a significant improvement in HR-QOL following kidney transplantation. This study also showed a lower perceived QOL in patients who experienced an acute rejection episode.22

To date, very few studies have compared humanistic outcomes of the various immunosuppressive regimens, but as additional agents become available this will become more relevant.

Application of Pharmacoeconomic Methods

Resource Utilization Methods

There are 2 primary methods for collecting data to be used in the economic evaluations of drugs. One way is to collect all the healthcare resources used for any given outcome. Clinical trials are often used as a way to collect major items of resource utilization such as hospitalizations and in-patient resources (drugs, lab tests, etc.). Some studies have attempted to collect actual financial data from each participating center; however, this method is limited by the interinstitution variability of charges/procedure or medication.23 A better method is to collect actual resource utilization data and then apply standard costs for the various items (i.e., Medicare reimbursement rates, etc.). This method also allows for standardization of charges, as if all of the procedures were performed in one center. Advantages of piggybacking these studies onto existing trials are that a large number of patients are randomized to the various treatments, the study has been powered to determine whether a statistically significant difference exists in predetermined endpoints, and the majority of the data are already being collected. If designed correctly, the financial or resource use data can be collected in a prospective manner. The major limitation of piggybacking pharmacoeconomic and outcomes research onto Phase III

Table 4: Variables in Cost-Consequence Modeling

<table>
<thead>
<tr>
<th>DIRECT MEDICAL COSTS</th>
<th>INDIRECT MEDICAL COSTS</th>
<th>CLINICAL OUTCOMES</th>
<th>HUMANISTIC OUTCOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug therapy</td>
<td>Noncompliance</td>
<td>Clinical disease features</td>
<td>Functional status</td>
</tr>
<tr>
<td>Physician visits</td>
<td>Work days missed</td>
<td>• rejection</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>Ancillary services</td>
<td>Family assistance</td>
<td>• infection</td>
<td>Satisfaction</td>
</tr>
<tr>
<td>• drug assays</td>
<td>Equipment/maintenance</td>
<td>• chronic rejection</td>
<td></td>
</tr>
<tr>
<td>• nephrotoxicity</td>
<td>Transportation costs</td>
<td>Diagnoses and cures</td>
<td></td>
</tr>
<tr>
<td>Hospitalizations/readmissions/LOS</td>
<td></td>
<td>Adverse effects</td>
<td></td>
</tr>
</tbody>
</table>

Adverse effects:
- nephrotoxicity
- hypertension
- hyperlipidemia
- steroid-related complications
- others
- Retransplantation
- Mortality

Table 4: Variables in Cost-Consequence Modeling

<table>
<thead>
<tr>
<th>DIRECT MEDICAL COSTS</th>
<th>INDIRECT MEDICAL COSTS</th>
<th>CLINICAL OUTCOMES</th>
<th>HUMANISTIC OUTCOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug therapy</td>
<td>Noncompliance</td>
<td>Clinical disease features</td>
<td>Functional status</td>
</tr>
<tr>
<td>Physician visits</td>
<td>Work days missed</td>
<td>• rejection</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>Ancillary services</td>
<td>Family assistance</td>
<td>• infection</td>
<td>Satisfaction</td>
</tr>
<tr>
<td>• drug assays</td>
<td>Equipment/maintenance</td>
<td>• chronic rejection</td>
<td></td>
</tr>
<tr>
<td>• nephrotoxicity</td>
<td>Transportation costs</td>
<td>Diagnoses and cures</td>
<td></td>
</tr>
<tr>
<td>Hospitalizations/readmissions/LOS</td>
<td></td>
<td>Adverse effects</td>
<td></td>
</tr>
</tbody>
</table>

Adverse effects:
- nephrotoxicity
- hypertension
- hyperlipidemia
- steroid-related complications
- others
- Retransplantation
- Mortality
multicenter trials (MCT) is the fact that the studies are conducted under highly controlled conditions (i.e., best-case scenario) designed to measure safety and efficacy of a regimen in ideal patients. High-risk patients who typically require more frequent monitoring and dosage adjustments are usually excluded during the screening process. These types of analyses would be better termed cost-efficacy, rather than cost-effectiveness analyses. It is not until the drug is used in the real-world setting that one can truly evaluate its effectiveness or cost-effectiveness. Additionally, the Phase III study has a stringent protocol for monitoring the drug therapy, and once practitioners learn how to use the drug, the monitoring frequency may be different than in the initial phase of the study. This latter factor makes it very challenging because pharmacoeconomic analysis is based on comparing a new medication to one with which practitioners have far more experience.

A pharmacoeconomic analysis performed on a Phase III MCT may find there is no additional cost-benefit with the new agent, but it is important to remember the learning curve effect may have an impact on subsequent costs. Another limitation is that the actual cost of the study drug and its monitoring is not known during a Phase III MCT, and in some trials this differential can be sufficient to sway the economic analysis in one direction or the other.

Comparisons of drug regimens, both for effectiveness and economics, should be conducted in large, randomized, prospective multicenter trials, and ideally performed 3 to 5 years after the drug has been approved, when everyone has experience using the new medication. Realistically, it is unlikely that the pharmaceutical industry would fund a study of such magnitude once the drug is approved and in widespread use.

**Pharmacoeconomic Modeling Techniques**

As described above, prospective pharmacoeconomic studies can be very complicated and take years to complete. Administrators typically want to know what impact a new medication, device, or procedure will have on their institution’s financial status in real time rather than waiting for actual results. Therefore, alternative strategies using statistical techniques are frequently used to predict future implications based on existing data and certain assumptions. Pharmacoeconomic studies commonly employ one or more of the following techniques to answer economic questions in a timely manner: modeling, decision analysis, or meta-analysis.

Modeling data have become a popular way of applying pharmacoeconomic analyses to various sources of data available within and outside healthcare organizations. Sources of data include medical records, financial and administrative databases, expert panels, randomized clinical trials, medical claims databases (e.g., Blue Cross and Blue Shield), government or other databases (e.g., Medicare, Medicaid, USRDS, UNOS), and private consultants. These types of studies typically use existing clinical and epidemiologic data to project the effect of a clinical, policy, or medication decision on a patient, population, or organization.

Advantages of modeling include that it is a relatively inexpensive and timely means of obtaining pharmacoeconomic data (i.e., utilizes existing data rather than repeating the study or collecting new data). Modeling can also serve as a bridge between efficacy data and effectiveness data, allowing one to populate the model with local or internal data rather than only using data from Phase III trials.

Modeling studies are also inherently disadvantageous, largely because they are approximations that are only as good as the assumptions made and the sensitivity of the model. Modeling also has the potential to introduce bias into its findings. It has been suggested that models can be designed to support any results desired by a researcher, sponsor, or decision maker. If a stakeholder sponsors the study, a degree of skepticism exists with any conclusions. It is also unlikely that a negative pharmacoeconomics study will be published if it reflects poorly on the sponsor’s product. Another limitation of modeling involves the quality of data incorporated into the model. The quality can vary greatly depending on the source and the rigor under which the data were collected. Finally, because of a lack of familiarity with modeling techniques, practitioners may question the value of data derived this way.

The easiest way to model one’s own data is to adapt an existing model to one’s specific institution by substituting outcomes data and institutional costs. This is not always possible because some of the...
models published in the literature do not provide adequate detail to allow you to perform your own calculations, nor do they always share the same key variables for your setting. Certainly, institutions with a heavy managed-care influence may have different priorities than those with less capitation.

**Clinical Decision Analysis**

Decision analysis is a modeling technique used under conditions of uncertainty. It quantitatively describes a problem in terms of multiple possible courses of action, probabilities that certain events and outcomes will occur, and the value of the expected outcomes resulting from those different courses of action. By combining the probabilities that events will occur with the value of each possible outcome, decision analysis determines which option to select to maximize the outcome of a given decision. A commonly used component of decision analysis is called the decision tree that incorporates the various outcomes. A variety of software packages exist to aid the clinician in performing decision analyses.

The main advantage of decision analysis is that it forces the user to structure a decision as well as identify the consequences of the possible decision outcomes. It is quantitative in that it forces the user to assign probability estimates and outcome valuations to identify the best outcome. Decision trees allow a therapeutic management problem to be separated into discrete manageable steps and work best for problems involving events or interventions that occur once over a short period of time. Furthermore, a treatment decision model can be based on the relative nature and degree of costs incurred under different treatment scenarios. Unfortunately, the majority of treatment decisions made today are not based on such models, largely because of the lack of suitable comparative published studies and because of the natural bias toward the selection of studies with positive findings for publication.

The main disadvantage of decision trees is that they can become very complex (i.e., multiple sequential branches) when trying to deal with events that occur repeatedly (e.g., acute rejection and infection) or over a prolonged period of time (e.g., chronic rejection). In these situations, it is better to use an alternative method, such as Markov modeling, which allows a patient to move from one condition to another.

**Markov Modeling**

Depending on the circumstances, a simple decision tree may not be adequate to address complex issues that can be characterized by the recurrence of various conditions. Conventional decision trees describe the various ways a group of patients in one state of health may end up in other states over a fixed period. Markov models, alternatively, focus on transitions among a number of possible health states (e.g., healthy, diseased, diseased with complication, and dead) during a series of time cycles. The general idea behind Markov modeling is that a patient can be in one state of health at any given time and that the patient’s health status can change from that state to another and in some situations back again, depending on a set of transition-related probabilities. Potential transplant “states,” in which the patient might be categorized, include well, rejection, CMV infection, other infection, chronic rejection, malignancy, renal failure, and death. Markov modeling is the best method, but other multistate models are reviewed elsewhere.

Advantages of Markov modeling include its utility for more accurately reflecting the various states in the clinical course of transplant patients. It can also be used to predict the impact of a change in immunosuppressive therapy on the expected survival and frequency of other events (e.g., what impact does a 50% reduction in rejection that results in a 50% increase in CMV have on survival and on long-term costs?). A limitation of this method includes using data from clinical trials, which may or may not provide information regarding new immunosuppressive regimens. For instance, it would be difficult to model the efficacy of different CMV prophylactic regimens in a sirolimus regimen if there are no data reporting the efficacy of a sirolimus-based regimen. One could make the assumption that the antiviral regimens are equally efficacious as in azathioprine or mycophenolate mofetil regimens, but this assumption would completely influence the outcome of the model. Similarly, if controlled trials are lacking for regimens currently in use, it is not possible to populate a
model with such data unless it is available in some other setting. Another limitation of the Markov model is the assumption that clinical events or states are mutually exclusive when, in fact, it is possible for a patient to have a rejection episode and a CMV infection at the same time or an acute rejection episode superimposed on chronic rejection. The model is only as good as the assumptions upon which it is based.

Markov modeling has primarily been used in transplantation for predicting trends such as number of patients requiring renal replacement therapy and transplantation in Denmark,28 Canada,29 and Australia;30 distribution of donor hearts to maximize recipient survival;31 progression of allograft vasculopathy after heart transplantation;32-33 and analyzing the cost of main clinical events after cardiac transplantation.34

Cost of Transplant-Related Complications

Acute Rejection

The cost of maintenance immunosuppression is high (Table 2). However, the cost of treating an acute rejection episode is also expensive if it does not respond to pulse steroid therapy. The cost to treat an episode of acute rejection is approximately $3,300 with a course of steroids and $14,500 with a course of antilymphocyte therapy, but may be even higher depending on the number of courses and duration of therapy needed to reverse the process.18,35

CMV Infection

CMV infection is well recognized for increasing length of stay and hospitalization charges following both kidney and liver transplantation.36-38 A number of economic studies have supported the use of ganciclovir16-40 in organ transplant patients, whereas valacyclovir was studied in another.41 Two of the studies supported antiviral prophylaxis only in the highest risk groups.39,41 The economic results from the valacyclovir study, using the French healthcare system perspective, were difficult to apply to U.S. centers since the length of stay was much longer than currently reported in this country.41

Das constructed a Markov model to compare the cost-effectiveness of different prophylactic strategies for CMV in a hypothetical cohort of 1000 liver transplant patients.42 Seven possible posttransplantation states of health were included in the analysis: healthy, those undergoing acute rejection, those with chronic rejection, patients with CMV infection but no disease, patients with CMV disease, those with CMV disease complicated by opportunistic infections, and the 7th state was death related or unrelated to CMV. The model was limited to the 1st year after liver transplantation to simulate the usual period of CMV-related morbidity and mortality and because of the lack of literature using CMV prophylaxis beyond this time period. Antiviral strategies included providing prophylaxis to all patients or to high-risk patients only (D-R-, steroid-resistant rejection, OKT3) and consisted of 5 different regimens (IV ganciclovir x 100 days, oral ganciclovir x 100 days, CMV immune globulin up to 16 weeks, acyclovir x 6 months, acyclovir x 3 months). In the initial analysis, all patients received some type of prophylaxis, with IV ganciclovir and oral ganciclovir identified as being the 2 best strategies. These 2 agents were then used in the 2nd stage of analysis to determine whether universal prophylaxis or selective administration to high-risk patients was preferable. Based on the incremental cost-effectiveness ratio, universal oral ganciclovir was the most favored strategy.

This outcome is not surprising considering the most effective strategies were the 2 different ganciclovir regimens (IV vs. PO); however, the model is limited in that it assumed IV ganciclovir would be administered for the full 3 months, which is more costly as compared with oral therapy for 3 months. Another limitation of the study is that some currently used combinations of CMV prophylactic agents (e.g., IV ganciclovir followed by PO ganciclovir, CMV-Ig in combination with ganciclovir) were not included. Similarly, the analysis of universal versus selective prophylaxis only compared these strategies against using no prophylaxis whatsoever, rather than against other contemporary regimens such as targeted preemptive therapy.

Steroid-Related Complications

Veenstra et al. used Markov modeling to predict the incidence and long-term cost of steroid-related side effects after renal transplantation.45 Data on the incidence of steroid-related complications (e.g.,
hypertension, posttransplant diabetes, peripheral bone fractures, avascular necrosis, cataracts) were obtained from the transplant literature and were limited to studies using cyclosporine-based immunsuppression. If data were not available in the transplant literature, other sources from the medical literature were used. A 10-year time frame was selected for capturing the costs of steroid-related side effects as it would reflect the average graft survival of a kidney transplant recipient. The most costly side effects were hypertension and posttransplant diabetes. The cost of treating steroid-related side effects over 10 years ranged from $2,500 to $7,500 per patient or $265,900 for the 50-patient cohort. Limitations of this analysis include the fact that not all steroid-related side effects were included such as lipid disorders and cardiovascular complications, hip fractures, glycemic control in patients with preexisting diabetes and diabetes-related complications, and stunted growth, nor were changes in quality of life related to steroids considered. These additional adverse effects may have increased the overall cost per patient.

This study highlights the importance of considering the costs and long-term consequences of immunosuppressant-related side effects. Certainly, as the economics of the various new immunosuppressive regimens are evaluated, it will be important to factor in the cost of using steroids when making decisions between equally effective but possibly steroid-free regimens.

Pharmacoeconomic Evaluations of Current Immunosuppressive Regimens

Economic studies evaluating immunosuppressive regimens have used the various procedures described above, although most have focused on the short-term impact of immunosuppressive therapies and limited their analysis to hospitalization costs and/or readmissions during the 1st year post transplant. Some have included out-patient data, but on a limited basis.

Cyclosporine (Sandimmune, Neoral)

The introduction of cyclosporine dramatically increased the cost of maintenance immunosuppression for transplant patients. However, previous studies have shown that the cost of adding cyclosporine to the regimen was offset by decreased readmissions for treatment of acute rejection during the 1st year after transplantation, making transplantation more cost-effective than dialysis. More recently, a number of economic analyses based on resource utilization have been conducted comparing the 2 cyclosporine formulations. Most were simple cost analyses that compared the direct medical costs of immunosuppressive therapy during the short term (e.g., 12 weeks to 1 year post transplant) after renal or hepatic transplantation. Two preliminary economic studies in Canada performed on the data from a stable conversion study and a de novo trial compared Neoral with the older cyclosporine (Sandimmune). These studies did not produce any statistically significant cost differences as resource utilization was similar in the 2 treatment groups, although there was a trend in favor of Neoral. Both studies enrolled a small number of patients, 30 and 41, respectively, and the duration was only 12 weeks. Another study in Europe enrolled 68 patients into a de novo trial, and these patients were followed for 12 months. From a societal perspective, potential savings of 27% from the use of Neoral was identified when compared with Sandimmune. In 3 other economic analyses, there was an overall cost advantage for Neoral in de novo livers of about 8% to 10% at 4 months, an advantage for Neoral versus IV in liver patients with respect to costs associated with acute rejection, and a cost savings from dosage reduction in a conversion trial at 6 months post transplant. A limitation of the above studies was that the studies were not primarily designed to test economic hypotheses. Most were not powered to detect a statistically significant difference in clinical outcome, and thus it is no surprise there were not statistically significant cost differences other than the savings produced by the differential pricing of Neoral versus Sandimmune.

Lewis et al. used Markov modeling to evaluate the cost-effectiveness of de novo Sandimmune cyclosporine versus the modified solution Neoral. The 2 Neoral cohorts were composed of 35 primary CAD renal transplant recipients participating in U.S. trial OLM 103 (Neoral-US) and an aggregate of 77 patients studied in European trials OLM 103, OLM 104, and OLM 105 (Neoral-EUR). Each tri-
al was a prospective, parallel group, randomized, double-blind comparative study of de novo Sandimmune (SIM) versus Neoral conducted during 1992 and 1993. Follow-up in each of the trial cohorts was limited to 12 weeks at the time of data analysis. The Sandimmune-treated patients consisted of the current controls participating in the U.S. de novo Neoral trial (SIM-US, n = 32) and a cohort of 4737 Sandimmune-treated, 1st-CAD transplant recipients selected from the U.S. Health Care Financing Administration (HCFA) databases (SIM-HCFA).

A Markov decision analytic model was constructed for each study cohort by assigning one of the following 4 health states to each patient: no previous rejection, one or more previous rejection episode(s), return to permanent dialysis because of graft failure, and death.53 Patients remained in the same health state or were transmitted to another health state at the end of arbitrarily selected, discrete-time intervals referred to as Markov cycles. The present model was run for 6 cycles, each of 15 days duration, to encompass an observation period of 3 months. Probabilities of rejection, graft loss due to rejection, graft loss due to other causes, and death were calculated for each 15-day Markov cycle. The cumulative probabilities of these events were then calculated and, together with itemized cost data, used to calculate the costs per functioning graft and per rejection-free clinical course for the first 3 months following transplantation.

Because the rejection rates within the various Neoral and Sandimmune cohorts varied so greatly and overlapped (32% to 45% and 26% to 61%, respectively), the data did not demonstrate a conclusive difference with respect to cost-effectiveness. The major limitations of the study were the small sample sizes in each of the de novo clinical trials, protocol-driven patient management and resource utilization in the clinical trial patients, and differences in European versus U.S. practice patterns that were not characterized in the de novo study databases. Another major limitation of the study was that the HCFA database was unable to distinguish between an antibody-treated versus corticosteroid-treated rejection episode, and a mean cost for all rejection episodes was calculated. Certainly the use of the actual cost for either polyclonal or monoclonal rejection therapy might have swayed the financial analysis.

**Tacrolimus**

Several studies have been conducted evaluating the short-term data comparing tacrolimus and cyclosporine based on studies in Europe and the United States.23,54-60 The majority of the studies focused on direct medical costs during the short term (e.g., 1st year) after renal or hepatic transplantation and were associated with immunosuppressive therapy and readmissions for acute rejection. In some of the studies, an overall cost advantage for tacrolimus of about 10% to 20% was reported,23,57,61 whereas others reported specific cost advantages (e.g., costs associated with acute rejection,23,57,61 immunosuppressive regimen,54,57,59 and subsequent rehospitalizations55,57,58). Most of the cost benefits of tacrolimus over cyclosporine were the result of lower rates of acute rejection reported with tacrolimus maintenance therapy.

It is always important to evaluate all of the data presented within an economic study. A good example of this is in a recent U.K. study that used a retrospective design to analyze resources used in the management of adult cadaveric renal transplant patients with Neoral or tacrolimus as primary immunosuppression.56 Eighty-nine patients with at least 6 months of follow-up were included in a cost analysis of hospital expenditures for that time period. The authors concluded that there were similar overall direct medical costs, with mean costs being 13,200 pounds for Neoral and 12,982 for tacrolimus patients; however, key factors including death, graft loss, and return to dialysis, which were higher in the Neoral group, were not included in the financial analysis.

Short-term and long-term benefits for tacrolimus were reported in a study by Gjertson and colleagues reviewing the data on 38,057 first cadaveric kidney recipients in the UNOS Kidney Transplant Registry from 1988 through 1994. One-year graft survival rates of 91.1% ± 1.3% versus 86.6% ± 0.2% were reported for tacrolimus versus cyclosporine, respectively. They estimated a significantly longer graft half-life of 14.5 years for the tacrolimus and 8.8 years for the cyclosporine...
If these figures are accurate, the implication is that the cyclosporine group will incur the extra cost of returning to dialysis or need for retransplantation 5 years sooner than the tacrolimus patients. Another interesting finding in this analysis was that 60% of the tacrolimus patients were reported to be steroid free by 1 year as compared with only 15% of the cyclosporine-treated patients. The graft half-life in the tacrolimus patients successfully withdrawn from steroids was 26 ± 10 years.

The primary limitation of this study was that only 24 (11%) of the centers contributed the tacrolimus patients. It is difficult to discern whether the improvement in graft survival is a reflection of the primary immunosuppressant or whether these patients, the majority of whom were steroid free, represent an immunologically privileged population, or whether steroids contributed to the decreased graft half-life seen with the other patients.

**Mycophenolate Mofetil**

An economic analysis based on the mycophenolate mofetil (MMF) multicenter clinical trial evaluated the costs of quadruple therapy involving induction, cyclosporine, corticosteroids, and MMF or azathioprine in the 1st year after transplantation. Treated acute rejection rates, graft failure rates, and medical care utilization data obtained directly from the U.S. trial were used as inputs to the economic analysis. Additional data were obtained from American Hospital Association annual reports (hospital per diem cost estimates), Medicare End-Stage Renal Disease program reports (annual dialysis and functioning graft expenditures), and literature-base patient preference (utility) estimates. Data from a U.S. quadruple therapy induction trial demonstrated a statistically and clinically significant reduction in the incidence of biopsy-proven acute rejection or treatment failure at 6 months (47.6% in the control group vs. 31.1% in the MMF 2-g treatment group \(P = 0.0015\)). The clinical results showed a much lower incidence of rejection, better graft survival, and no difference in the incidence of opportunistic infections with MMF therapy. Even though MMF was more expensive than azathioprine, the cost of MMF was offset by the lower 1st-year treatment costs for rejection, dialysis, and graft failure. MMF was deemed to be more cost-effective from a societal perspective than azathioprine, and even in the worst-case scenario, with sensitivity analysis applied, MMF was cost-neutral at the end of 1 year.

Two other economic analyses with MMF were performed in Canada but provided conflicting data, with one reporting slightly higher costs with MMF therapy and the other finding MMF to be more cost-effective. Limited data are available as both were only reported in abstract form. Three other single-center analyses reported early economic benefits from the health system perspective, primarily related to the decreased incidence of rejection 3 to 6 months posttransplantation and less need for expensive antilymphocyte therapy.

**Sirolimus**

Limited pharmacoeconomic data are available for sirolimus. A recent abstract described an economic analysis using Medicare claims data for the 1st year charges from the recent U.S. sirolimus safety and efficacy trial. The analysis showed lower inpatient and physician/supplier charges ($4600) for the sirolimus 2 mg/day arm as compared with azathioprine; however, the cost of the study drugs was excluded.

**Induction Regimens**

Much controversy has existed regarding the benefits of induction therapy as the randomized trials have failed to show improved allograft survival. Szczech et al. recently conducted a meta-analysis of these trials, which showed a benefit of induction at 2 years, particularly among presensitized patients, and in the latter population, the patients continued to have a benefit at 5 years.

This controversy also exists for pharmacoeconomic analyses of the various products as conflicting data exist for the comparative studies and reflect the differences that may occur at single centers versus pooled data from multicenter trials.

Shield et al. compared the cost of induction therapy with OKT3 versus no induction therapy with cyclosporine, azathioprine, and prednisone by modeling clinical trial results with financial data from separate sources. Cost estimates were based on results from a 5-center randomized trial.
paring OKT3 induction with conventional triple drug therapy in 207 patients. Financial data were obtained from the National Cooperative Transplantation Study, the Medicare Provider and Analysis Review database, and other sources. The comparative measures included costs incurred between transplantation and graft failure, the effectiveness of the 2 regimens as defined by length of graft survival, and cost-effectiveness ratios through 5 years of observed follow-up, and modeled beyond 5 years by assuming a graft failure rate of 4% annually. The authors concluded that the initial cost of the OKT3 induction therapy was almost offset by savings associated with a lower acute rejection rate and a trend for better graft survival. However, depending on which parameter is evaluated, one could conclude that OKT3 is more expensive, less expensive, or cost-neutral. Another single-center study reported favorable results and improved cost-effectiveness with a shorter course of OKT3 therapy, but they did not perform a formal economic analysis.72

Schommer et al.73 performed a retrospective analysis comparing the economics of ATG and OKT3 in a retrospective, multicenter study using charge data obtained from the HCIA “Clinical Pathways Data Base.” Five hundred fifty-two patients who had received either OKT3 or ATG were selected from 22 hospitals. The authors concluded that the increased pharmacy charges for ATG were partially offset by reductions in ancillary charges. In a subsequent publication, the authors pointed out the limitations of using secondary databases and that significant variations between hospitals’ clinical practices and charging policies made interpretation of the results difficult.74

Brennan et al. conducted a retrospective analysis of their single-center experience of 183 patients receiving induction therapy with either ATG or OKT3.75 There were some demographic differences between the 2 groups as the ATG patients were older, which might have contributed to the lower incidence of rejection, but more extended donors were also used in that group. The 1-year posttransplant rejection was lower for ATG (34% vs. 47%) than for OKT3, and graft survival was better in the ATG group (93% vs. 85%). The overall hospital-related costs for ATG ($39,937 ± $17,014) and OKT3 ($42,850 ± $20,923 for OKT3) were similar.

Schnitzler et al.76,77 demonstrated cost savings for thymoglobulin as compared with ATG in the treatment of acute rejection. This pharmacoeconomic study was conducted from the perspective of Medicare and performed on the data from 163 patients enrolled in the randomized double-blind 25-center trial evaluating the safety and efficacy of these agents in reversing acute rejection. The study focused on the first 90 days following initiation of rejection therapy and assessed differences in immunosuppression, therapy for refractory rejection, CMV treatment, and return to dialysis, and complications requiring hospitalization were included in the analysis. Thymoglobulin was associated with a significantly lower cost (overall $5277 savings) during the 90 days posttherapy, with a cost difference of $7133 in recipients of cadaveric donors. Savings ranged from $6,581 to $12,509 in other high-risk subpopulations. It is important to note that the cost of both study agents was excluded, as thymoglobulin had not yet been priced and inclusion of this information could change the savings differentials.

Other Methods to Reduce the Cost of Immunosuppressants

Other efforts that have been used to reduce the costly nature of immunosuppressants include the intentional administration of interacting medications (e.g., ketoconazole, diltiazem, itraconazole, erythromycin) or food products (e.g., grapefruit juice).78-80 These strategies for reducing dosages, necessary to achieve therapeutic concentrations, are dependent on the competitive inhibition of cytochrome P-450III A4 enzymes and p-glycoprotein to improve the absorption of agents such as cyclosporine, tacrolimus, and sirolimus. A dosage decrease and cost savings can be achieved by these strategies, but the added monitoring costs need to be considered.

Summary

As more and more immunosuppressive agents are introduced to the market, practitioners need to scrutinize both the reported clinical results and the subsequent economic analyses. A number of so-called pharmacoeconomic studies have been published in the literature, but most are limited by
REFERENCES

1. 11999 Mean Local Standard Acquisition Charges for All Organ Types. 1999 Association of Organ Procurement Organizations (AOPO) Annual Report.
2. Estimated US average billed charges per transplantation as of July 31, 1999. Millman and Robertson, Inc.


“Engineering” Myoblast Transplantation

Daniel Skuk and Jacques P. Tremblay

Myoblast transplantation (MT) designates the intramuscular implantation of myogenic cells as a treatment for muscle diseases. As a therapeutic tool, MT can act in 2 complementary manners: It can be a vehicle for a normal genome and it can increase the myogenic capacity of the host muscle. Although many experiments in rodents demonstrate these properties, the experiments in nonhuman primates allow for a better definition of the parameters that allow for making MT an applicable strategy in humans. In the present review, special attention is given to the clinical possibilities of MT. Two challenging factors are especially analyzed: the strategy of cell delivery and the control of rejection. The 3 issues that the authors identify as requiring further study to introduce improvements in MT design are intramuscular donor-cell migration, early donor-cell survival, and methods to avoid allograft rejection (development of specific tolerance or autotransplantation of genetically corrected myoblasts).

Dystrophin:
A fibrilar protein that connects sarcomeric actin with a complex of proteins related to the sarcolemma.
skeletal muscle is to generate voluntary mechanical work, which is done by polynucleated syncytia called myofibers (Fig. 1). Myofibers are long enough to join the points that must be reached for mechanical work, and parallel to each other, allow a sum capacity to generate force. Myofibers are joined by a delicate connective tissue (the endomysium), are grouped into fascicles joined by the perimysium, and are surrounded by the epimysium. This connective network ensures the coordinated work of the ensemble.

Since myofibers are differentiated syncitia with a highly specialized structure, they cannot enter mitosis and, thus, cannot be expanded in culture or used for transplantation. For MT, a precursor myogenic cell capable of proliferating in vitro and differentiating into myofibers is needed. During postnatal life, the skeletal muscle ensures growth, hypertrophy, and regeneration of necrosed myofibers through the presence of mononucleated myogenic stem cells named “satellite cells” (for a review, see ref. 15). The denomination of “satellite” is due to their placement, occupying a depression on the periphery of the myofiber, lying between the sarcolemma and the basal lamina (Fig. 1). An injury causing myofiber necrosis stimulates satellite cells to be drawn out of their quiescent state, proliferating and fusing, either to fill the defect produced by segmental necrosis (Fig. 2) or to restore an entire myofiber after total necrosis. Satellite cells can be isolated enzymatically from muscle and can be proliferated in vitro (Fig. 1), maintaining their capacity to fuse and differentiate into myofibers. As mononucleated and undifferentiated cells with the capacity to differentiate in skeletal muscle, these cells are referred to as “myoblasts.”

The transplantation of these cells into host muscles has 2 complementary properties: Donor myoblasts are vehicles of normal genome, and they can increase the myogenic capacity of the host muscle.

Donor Myoblasts as a Vehicle of Normal Genes

After fusing with host myofibers, donor myoblasts induce “gene complementation”; that is, myofibers will express genes of both donor and host nuclei, allowing the expression of the protein that is mutated in the host nuclei (Fig. 2). These myofibers are referred to as “hybrid” myofibers. Gene complementation was demonstrated after MT in myopathic mouse models. MT in the mdx mouse, a model of DMD, led to dystrophin expression in host myofibers.16-19 There is evidence that this expression of donor-dystrophin protects mdx myofibers from the pathological process.16,20,21 In the dy/dy mice, a model of congenital dystrophy with lack of merosin, MT leads to hybrid fibers expressing merosin.22 MT in the SJL/J mice, a model for limb-girdle muscular dystrophy with lack of dysferlin, restored dysferlin expression in hybrid myofibers.23

Donor Myoblasts Increase the Myogenic Capacity of the Muscle

A characteristic of DMD is the progressive exhaustion of satellite cells after recurrent cycles of degeneration-regeneration. The injection of donor myoblasts can act as reinforcement to the myogenic capacity in these muscles. Restoration of muscle mass and force after MT was observed in many rodent experiments.24-27 Another potential benefit of MT would be the capacity to reconstitute a pool of donor satellite cells able to participate in later regeneration. Donor myoblasts remaining as quiescent satellite cells were observed in biopsies of DMD patients that participated in an MT clinical trial.28 In rodents, there is evidence that some donor myoblasts persist as precursors able to participate in later muscle regeneration,29,30 specifically, as functional satellite cells.31

The Strategy of Cell Delivery

The most efficient method to deliver donor myoblasts to muscles is direct injection. Intramuscular injections ensure sufficient quantities of donor cells into the host tissue. They produce muscle injury, allowing myofiber regeneration (that incorporates efficiently the donor cells in the preexisting myofibers), and the breaking of endomysial tubes (an event that could allow the movement of the donor myoblasts into them). Two parameters must be defined for an efficient strategy of donor-cell injection: the optimal distance between injections and the optimal number of cells to be delivered per injection trajectory. These parameters will depend on a previous definition of the objective to be reached.
Figure 1. (1) As a treatment of muscular dystrophies, the target of MT is the striated skeletal muscle. (2) Skeletal muscles are composed by long syncytia (myofibers) parallel-arranged. (3) Satellite cells, the quiescent myogenic stem-cells situated at the periphery of the myofibers, are the principal source of donor cells for MT. (4) Satellite cells can be isolated from muscle and proliferated in vitro as myogenic mononucleated precursors (myoblasts). (5) For monkey experiments, these myoblasts are labeled in vitro by retroviral transfection with the LacZ gene and injected in a biceps brachii using a grid to define the inter-injection distance. (6) A cross section of the whole bicep shows high percentages of myofibers expressing β-galactosidase (dark staining). (1: detail of Rembrandt's Anatomy Lesson of Dr. Tulp, 1632.)
Figure 2. (1) In monkeys, donor myoblasts are injected by parallel injections, perpendicular to the muscle axis. (2) The needle produces myofiber damage during introduction (2a), and cells are delivered in the injection trajectory during needle withdrawal (2b). (3) The injection produces myofiber damage (3a), triggering a process of segmental regeneration in the presence of donor cells. Muscle regeneration involves proliferation and fusion of myogenic cells (3b), which in this case is done with the participation of donor and host cells. After fusion (3c), donor-cells are incorporated in a “hybrid” myofiber (3d). The expression of those proteins present exclusively in the donor cells (e.g., dystrophin in the case of DMD) is obtained in a region of the hybrid myofiber: the nuclear domain of the donor-nuclei (3d). (4) One month after MT, expression of the reporter protein is observed in those myofibers reached by the injections. (5) This is evident in cross sections of monkey muscles, where the trajectories of myoblast-injection are seen as tracts of β-galactosidase-positive myofibers (in dark). Original magnification (5): 25X.
Defining an Objective
The ultimate objective of MT is to improve the muscle function in severe myopathic patients and to ensure an acceptable quality of life. Since there are no monkey models of myopathies, experiments to test the functional effects of MT cannot be conducted in nonhuman primates. However, injection of β-galactosidase-labeled myoblasts in normal monkeys allows for an understanding of the parameters that produce high numbers of hybrid myofibers in the muscles. To extrapolate these data for DMD, it is necessary to know the percentage of dystrophin-positive fibers that must be obtained for a clinical effect. This can be estimated by the observations in DMD carriers. DMD carriers have a mosaic of dystrophin-positive and dystrophin-negative myofibers and can be asymptomatic or exhibit different degrees of severe to mild myopathy. Some authors have observed a correlation between the percentage of dystrophin-positive fibers and the clinical phenotype, as illustrated in Figure 3. It can be supposed schematically that less than 25% of hybrid fibers (as a result of MT in a DMD patient) may not significantly ameliorate the clinical picture, between 50% and 80% may significantly slow down the clinical evolution (leading to a mild myopathy), and between 80% and 100% will stop the evolution. Since most of our recent monkey experiments reached the second category (Fig. 3), this model allows us to advance toward the goal of setting parameters for clinical MT. It must be noted that some differences can be expected when comparing β-galactosidase and dystrophin expression, considering the different nuclear domains of both proteins.

The Inter-Injection Distance
The distance between injections may be determined by the efficacy of each individual injection, that is, the volume of muscle that will express the reporter gene introduced by the donor myoblasts (e.g., dystrophin or β-galactosidase) after a single injection. The more efficacy of a single injection, the more distributed the injections can be to reach the predetermined objective. The efficacy of an individual injection depends on

1. the capacity of donor myoblasts to migrate and fuse with distant myofibers,
2. the extent of the muscle damage around injections, and
3. the length of the nuclear domain for the protein restored.

The intramuscular injection of myoblasts in monkeys (the donor-cells homogeneously delivered during the needle withdrawal) produces defined tracks of hybrid myofibers (Figs. 2, 3, and 4). This indicates that donor myoblasts are incorporated mostly by myofibers along the injection trajectory. These observations concur with mouse experiments that show primary cultured myoblasts do not migrate through nondamaged muscle. Since a single myoblast injection produces a defined track of hybrid myofibers, the percentage of hybrid myofibers in a muscle section depends on the density of these tracks, and, therefore, on the density of injections. In monkey experiments, an inter-injection distance of 1 mm resulted in 25% to 70% of myofibers expressing a reporter gene present in the donor myoblasts, whereas an inter-injection distance of 2 mm produced only 6% to 12% of myofibers expressing the reporter gene (Fig. 3).

The inter-injection distance could be increased if the volume of muscle expressing the reporter gene following a single injection is increased. This potentially could be achieved by

1. increasing the capacity of the donor myoblasts to migrate and fuse with distant myofibers,
2. producing more tissue damage to provide more regenerating myofibers able to incorporate the donor myoblasts and initiate more extracellular matrix breakdown, and
3. increasing the nuclear domain of the therapeutic donor-protein.

Increasing the Intramuscular Migration of Donor Myoblasts
Our experimental data support the idea that inducing the secretion of metalloproteinases (the enzymes that migrating cells, such as leukocytes, use to degrade the extracellular matrix) could increase the migration of myoblasts through the muscle tissue. Incubation of donor myoblasts with concanavalin A (an inducer of metalloproteinase expression) increases the dissemination of donor myoblasts in the host muscle.

SYNCYTIA
Plural of syncytium: a multinucleated mass of protoplasm produced by the merging of cells.
Increasing the Fusion of Donor Myoblasts with Host Fibers

A larger number of regenerating myofibers increases the opportunities of donor myoblasts to be incorporated into most host myofibers. One of the most frequently used methods to produce this effect was the injection of notexin, a potent myotoxic phospholipase from the venom of the Australian tiger snake *Notechis scutatus scutatus*. Inhibiting the participation of host satellite cells in muscle regeneration will also favor donor myoblast incorporation after muscle damage, and the most used method to obtain this effect in experimental MT is ionizing radiation. Interventions that combined myonecrosis and host satellite cell elimination, such as cryodamage, were also used to improve MT in rodents. Although it is doubtful whether these methods can be applicable to improve MT in humans, this must ultimately be evaluated in a cost-benefit balance, that is, how much a difficult intervention will benefit the patient.

The Number of Donor Myoblasts Delivered per Injection Trajectory

It was shown that the number of hybrid myofibers increased (within a given range) along with the number of donor myoblasts until reaching a plateau. Monkey experiments showed that within a given range, the success of transplantation increased with the number of myoblasts injected (for a similar inter-injection distance), whereas a plateau is reached at higher donor-cell concentrations. Defining the optimal number of myoblasts...
Figure 4. Three main factors condition the clinical characteristics of MT, meriting further studies to improve this technique. Schematically, we represented them as early factors, affecting donor cells before fusion, and late factors, affecting donor cells after fusion: (1) The capacity of donor cells to migrate from its site of implantation and to fuse to myofibers distant from the site of injection. Since this capacity is insignificant after injecting primary-cultured myoblasts in nonseverely damaged muscles, this factor implies that donor cells must be implanted in each site where they must fuse with host myofibers. In the practice, injections must deliver donor cells homogeneously during needle withdrawal through all the width of the muscle, and injections must be placed very close from each other. Interventions aiming to improve the fusion of donor myoblast with myofibers distant from the site of injection will allow to increase the inter-injection distance. (2) The early survival of the donor-cell population after transplantation. This factor is still not well characterized, but many studies suggested that a large percentage of the donor cells die early after their implantation (2a). Our group has proposed that this is basically determined by infiltrating neutrophils (2b), although more evidence is still necessary. It is suggested that proliferation of the surviving donor cells (2c) compensates the early cell death, contributing to the donor-cell survival. Research to characterize further this factor, aiming to improve donor-cell survival, may allow to lower the number of donor cells to be injected. (3) The acute rejection of donor cells and myofibers expressing donor antigens. Control of acute rejection is achieved with immunosuppressive drugs, but with the consequences of toxic effects. Interventions in this field must be directed to avoid acute rejection by developing specific tolerance or by autotransplantation of genetically corrected myoblasts.

The microphotographs show the morphological expression of these factors: (1) A cross section of a monkey muscle transplanted with LacZ-transfected myoblasts shows that donor myoblasts have been incorporated only in the myofibers reached by the injections (the arrow shows the orientation of the original injections). (2) In a mouse muscle, a pocket of donor myoblasts (between arrowheads) is observed 6 h after implantation. Alizarin red staining shows extracellular-fluid penetration both in myofibers damaged by the injection (arrow) and in donor cells of the implant. (3) One month after myoblast allotransplantation in a monkey receiving low immunosuppression, the muscle shows intense lymphocyte infiltration between myofibers and around vessels (arrow). Original magnifications: 25X (1), 400X (2), and 200X (3).
to be delivered per injection will allow for the best MT results, without wasting large quantities of donor cells. Although further work to define this parameter will be necessary, recent monkey experiments showed that 50% to 70% of hybrid myofibers were observed after injecting 10^6 β-galactosidase-labeled myoblasts in 1 cm³ of muscle, using an inter-injection distance of 1 mm.

**Can the Number of Injected Myoblasts Be Reduced?**

There is some evidence that the intramuscular injection of donor myoblasts can be followed by a rapid donor-cell death.43-46 (Fig. 4). At present, we cannot say that the problem of early survival of the donor myoblasts is well understood. Indeed, the success of MT in monkeys11,12 shows that the early survival of the donor myoblasts is not a limiting factor for MT in primates. In spite of this, a better definition of this problem may help to lower the number of myoblasts to be injected.

**Can Myogenic Cells Be Systemically Delivered?**

The possibility of delivering myogenic cells through the blood stream was tested because of the obvious advantage of this route: accessibility to many muscles by a single injection, including important muscles (such as the diaphragm), which are inappropriate for direct injections. Injection of primary cultured myoblasts, intraperitoneally and intravenously, was negative, even when extensive muscle damage was produced to favor the incorporation of the donor myoblasts.47 Some success was obtained by intra-arterial administration of myoblasts from a cell line, but only when the host muscle was mechanically injured.48 Extracorporeal circulation was also used to infuse donor myoblasts directly into muscles, but the success of this approach was dependent also on inducing muscle injury.49 The possibility of delivering pluripotent stem cells with myogenic capacities by the blood stream (such as unfractionated bone marrow,50 haematopoietic stem cells,51 and muscle-derived stem cells52) was tested, but it was consistently observed that donor cells fuse with host myofibers after muscle damage. Therefore, if host muscles must be extensively injured to incorporate donor myogenic cells infused in the blood stream, systemic cell delivery will not be more advantageous but, indeed, more complex than direct intramuscular cell injections.

**Control of Rejection**

Even if an efficient donor-cell delivery is achieved, donor cells and hybrid myofibers must survive the mechanisms of allograft rejection. In organ transplantation, 3 types of rejection menace the survival of the graft: hyperacute, acute, and chronic. Hyperacute rejection occurs within minutes to hours after transplantation and is dependent on complement activation by preformed antibodies. This problem is usually avoided by the routine pretransplant screening of antidonor alloantibodies. In acute rejection, alloantigen-reactive cytolytic T-cells destroy the graft by a mechanism implicating MHC recognition on the donor cells. Chronic rejection, although not examined in long-term MT experiments, is basically produced by different vascular endothelial injuries in the graft, and it is not clear whether it can be produced in the recipient vessels of muscles implanted with myoblasts.

**Acute Rejection in MT**

Although MHC expression is not observed in normal mature myofibers, they exhibit MHC expression during inflammation, muscle regeneration, and in DMD.53,54 MHC is also expressed in myotubes.55 Acute rejection following MT is well documented and was observed in mice,41,56-58 dogs,59 and monkeys5-12 (Fig. 4). Infiltration by CD8+ and CD4+ lymphocytes was observed in host muscles after allogeneic MT in immunocompetent, nonimmunosuppressed mice.56 Expression of IL-2 receptors, Th-1 cytokine, and granzyme B confirmed that these infiltrating cells are activated lymphocytes.60,61 Rejection due to minor antigens was also observed after syngeneic MT from males to females.52 Another factor that can trigger immune reactions is the expression of the therapeutic protein that is absent in the host (e.g., dystrophin in DMD patients), although whether or not this factor triggers rejection was controversial.63,64

**Immunosuppression**

The degree of control of the humoral response after MT in mouse experiments varied among differ-
Figure 5. Transplanting with myoblasts many regions of the body would be especially challenging because of the anatomical complexity and the presence of many noble structures (such as in the neck). The forearm is represented because of the complex superposition of muscles with peripheral nerves and vessels. The figure summarizes the possible stages in a hypothetical modus operandi of a device for whole-muscle stereotaxic cell delivery, aiming to reach the forearm from the anterior and posterior sides: (1) The limb is scanned. (2) The operator individualizes the different anatomic structures. (3) The operator selects the targets for cell delivery and the anatomic structures that must be avoided. (4) The automated device for cell injection delivers the donor cells only in selected regions not blocked by protected structures.
ent immunosuppressive agents.\textsuperscript{65} Cyclophosphamide showed negative results in mice, and it was suggested that this drug kills the transplanted cells because of its antiproliferative properties.\textsuperscript{66} Cyclosporine A was administered in some MT clinical trials\textsuperscript{4-6} and was effective in mouse experiments.\textsuperscript{1,57} Nevertheless, cyclosporine A was less effective than sirolimus in controlling the humoral response against allografted myoblasts.\textsuperscript{66} Sirolimus was very effective as an immunosuppressant for MT in mice,\textsuperscript{19} although the best success was obtained using tacrolimus immunosuppression.\textsuperscript{17} Under tacrolimus immunosuppression, up to 95\% of the myofibers in \textit{mdx} mice expressed dystrophin after MT.\textsuperscript{17} Tacrolimus was effective in controlling the acute rejection after MT in monkeys\textsuperscript{9-12} and was observed up to 1 year following transplantation in this model.\textsuperscript{12} Control of acute rejection after MT was also obtained using monoclonal antibodies directed against lymphocyte adhesion molecules.\textsuperscript{67}

\textbf{Potential Strategies to Avoid Immunosuppression}

Although the new immunosuppressive drugs have significantly improved the results of clinical transplantation, these drugs have side effects. The long-term need for administration of immunosuppressants increases the risks of life-threatening infections and cancer. An important field in MT research is the development of strategies to avoid long-term immunosuppression, either development of tolerance or autotransplantation of cells genetically corrected in vitro.

\textbf{Development of Tolerance}

Although immunologic tolerance to allogeneic grafts can be induced with different strategies in mice, it was more difficult to achieve in nonhuman primates.\textsuperscript{19} The 3 main strategies tested in the nonhuman primate model were mixed allogeneic chimerism, T-cell depletion, and costimulation blockage (for a review, see ref. 13). Development of immunologic tolerance in the context of MT is currently being studied in our laboratory. We showed that a short course of anti-CD154, in combination with donor-specific transfusion, prolonged the survival of the hybrid myofibers, although a slow rejection was observed at long periods.\textsuperscript{61}

\textbf{Autotransplantation of Genetically Corrected Myoblasts}

Autologous MT requires genetic correction of donor cells in vitro. Both the human mini-dystrophin and the full-length dystrophin genes were transferred with adenoviral vectors in vitro to \textit{mdx} myoblasts, and these genetically corrected myoblasts produced dystrophin-positive myofibers after transplantation in \textit{mdx} mice.\textsuperscript{69,70} The mini-dystrophin gene was also introduced in human DMD myoblasts, and these genetically corrected DMD-myoblasts were transplanted in SCID mice, producing myofibers expressing the human mini-dystrophin.\textsuperscript{71} However, as explained before, the introduction of dystrophin could be potentially immunogenic in DMD patients.\textsuperscript{63,64}

In addition to the problems of efficiently introducing the correct gene in the donor cells, another difficulty of this approach is the limited capacity of autologous DMD myoblasts to proliferate in culture. As a consequence of recurrent cycles of myofiber degeneration-regeneration, senescence of DMD myoblasts in culture occurs early.\textsuperscript{72-74} Some strategies are being tried to increase the proliferative capacity of DMD myoblasts. One of them is the expression of the SV40 large T antigen, which delayed senescence in myoblasts but failed to induce cell immortality.\textsuperscript{72} The other one was the introduction of the telomerase gene, although telomerase expression alone did not significantly extend life span of human myoblasts.\textsuperscript{73} It was the coexpression of telomerase and T antigen that allowed DMD myoblasts to divide more than 55 doublings, preserving the ability to fuse and differentiate.\textsuperscript{74}

\textbf{Is MT Attainable in Humans?}

A clinical MT strategy based on our monkey observations implies some complexities.

1. Each injection must be as efficient as possible; thus, it must be made across the whole muscle, delivering the donor myoblasts homogeneously throughout its trajectory.
2. Injection trajectories must be placed very close to each other.
3. Delivery of donor myoblasts in subcutaneous tissue and nonmuscle organs must be avoided.
4. Some anatomic structures (e.g., peripheral nerves and large vessels) must be spared from repetitive needle damage.
Taking into account the complexity of efficiently injecting myoblasts through 40% to 50% of the body with the premises exposed, MT becomes a challenging task. If we desire to move to clinical applications, we must propose a method extrapolating from the experience obtained in a single monkey muscle to most of the skeletal muscle system of a human. In fact, if the previous premises will be the only ones allowing a significant genetic correction in the muscles of dystrophic patients, we believe that the future of MT will be determined by the use of automated systems for whole-muscle stereotaxic cell-delivery. This is because making 100 injections per cm² of muscle surface may be more rapidly and efficiently performed by an automated device capable of injecting simultaneously with several needles. Furthermore, to reach as much muscle tissue as possible, avoiding donor-cell delivery in nontargeted tissues and avoiding repetitive lesions of other organs, requires accurate topographical precision for cell delivery (even if the inter-injection distance can be increased). The principle of stereotaxic cell delivery to muscles (as we envision this possibility) is illustrated in Figure 5. The principal factor of risk associated with repetitive and close muscular injections is the extensive muscle damage associated with the release of intracellular metabolites (mainly myoglobin and potassium), although this factor can be managed by controlling the volume of muscle injected per MT session. As in other fields of medicine, MT technology (and possibly other types of cell transplantation) may need the interdisciplinary cooperation of different specialties, not only immunology and molecular biology but also medical imaging, data processing, and robotics. Whether this complex technology will be developed may depend on the results of better-planned MT clinical trials and also on a cost-benefit balance compared with the potential success of other experimental approaches to the treatment of muscular dystrophies.

References


Transplantation tolerance remains the goal of clinical transplantation and the focus of a large amount of research. Despite anecdotal reports,1 a rational scheme for reliably inducing and maintaining clinical transplant tolerance has not been defined. Nonetheless, clinicians and researchers realize the need for designing and testing assays capable of defining the immunologic fingerprint of a tolerant state. At the recent Transplant 2001 meeting, the 2nd joint meeting of the American Society of Transplantation (AST) and American Society of Transplant Surgeons (ASTS), we were charged with moderating a workshop entitled, *The Tolerance Assay: Where Are We Now?* A very lively discussion resulted in the consensus, which we report here.

Prior to the workshop, we both agreed that one of the great challenges in developing a reliable tolerance assay is the lack of a universal definition of “tolerance.” The ability to measure tolerance using a laboratory test clearly requires a “gold standard” definition of tolerance itself. The workshop started with a fairly textbook definition of transplantation tolerance:

> The normal function of a transplanted organ, without exogenous immunosuppressive therapy, in the absence of a pathologic donor-specific immune response, but accompanied by an otherwise fully competent immune system.

The 60 to 100 people attending the workshop initially agreed that this was a reasonable definition. However, upon probing a bit further, there were many different opinions on the details of that definition. In an effort to facilitate the discussion, we used a series of questions originally formulated by Dr. Charles Orosz regarding how to best model human tolerance in rodents. It was thought that developing criteria for defining tolerance in rodents, where controlled testing can be performed, would be easier than initially attempting to define tolerance in humans. The questions are printed below, and the resulting consensus was quite surprising to us all.

To model human allograft tolerance, allograft tolerant mice (or rats):

(a) must, (b) should, (c) may, (d) may not, or (e) should never:

1. Display micro-chimerism,
2. Display altered T cell responses to donor antigen,
3. Accept a similar allograft without further therapy,
4. Accept a donor-matched skin graft,
5. Display chronic-rejection like histologic changes,
6. Display persistent inflammation within the graft,
7. Require intermittent immunosuppression,
8. Lose an allograft 200 to 300 days after transplantation,
9. Require preparative irradiation,
10. Display clonal deletion of graft-reactive T cells,
11. Produce donor-reactive IgG,
12. Require preparative injection of donor bone marrow.

**Results**

1. A few individuals thought microchimerism must or should be present. In contrast, the majority thought that micro-chimerism may or may not be displayed.
2. All participants thought that donor-reactive T cell responses should be altered, either through deletion of the reactive cells or through regulation (suppression, cytokine immune deviation, etc).
3. All participants thought that a 2nd allograft of the same type must/should be accepted without further treatment.
4. Most participants thought that donor-matched skin graft should be accepted. This is the ideal situation, in which the strongest stimulus is accepted.
5. All participants thought that chronic rejection-like histologic changes should never be present.
6. Mononuclear cell infiltration within the graft is acceptable, but tissue damage resulting from that infiltration is never acceptable. One point made was that the presence of an infiltrate may be related to the underlying disease, rather than due to alloantigenicity.
7. Participants thought that intermittent immunosuppression may be used, although the absence of intermittent immunosuppression is preferable.
8. Graft loss after 200 to 300 days may be acceptable, especially given that the average life span of a mouse is not much more than 365 days.
9. Participants did not think that preparative irradiation must be required, but its use is acceptable as long as the re-
cipients are immuno-competent after transplantation.
10. Clonal deletion of graft-reactive T cells is considered necessary by some participants, but not by the majority. Most participants thought that regulation or blockade of the donor-reactive T cells is sufficient.
11. In general, donor-reactive IgG should not be present, according to most participants. However, a significant proportion raised the issue of protective antibodies.
12. Preparative injection of donor bone marrow is not required, but is acceptable.

Overall, the consensus opinion was much more vague than the original proposed definition. An animal model for graft tolerance that best models tolerance in humans will exhibit long-term, but not necessarily indefinite, graft survival and will exhibit no evidence of chronic histopathologic changes attributable to chronic rejection. The animals should accept a 2nd allograft of the original donor strain and, ideally, accept a skin graft of the original donor strain. In terms of immune function, donor-reactive T cells and alloantibodies should either be absent or should exhibit a protective or regulatory phenotype.

The participants were then asked an additional question: To be considered a viable model of the human allograft tolerance, does the rodent model need to be operative in one inbred strain, in several inbred strains, or in outbred mice? The consensus was that ideally the model would be operative in outbred mice but that showing the tolerance using at least 2 disparate recipient strains was sufficient (for example, BALB/c and C57BL/6).

The group next considered whether any of the existent rodent models actually meet all of these criteria. The 3 models most discussed were those introduced and initially studied by Megan Sykes (irradiation and preparative bone marrow transplant, reviewed in ref. 2) and by Kathryn Wood (DST and anti-CD4 antibody treatment), along with Anita Chong’s modification of the protocol originally described by the Larsen/Pearson group at Emory (anti-CD154 antibody and donor bone fragment at the time of transplantation). In the latter system, femur fragments are placed under the kidney capsule on day 0 and antibody is administered on days 0-3, 5, 7, 9, 11, and 13 relative to heart transplantation. A challenge with a 2nd heart is performed on days 60-90.

The tolerant animals accept donor, but not 3rd party, grafts. The tolerance is associated with a peripheral loss of donor-reactive IFNγ production by T cells, but central T cell deletion does not occur. Allograft tolerance has been induced by this approach in inbred mice (C57BL/6) and in mice on a mixed background (129 × B6 × DBA/2). The Chong adaptation at this time appears to meet the criteria of only peri-operative immunosuppression: an absence of alloantibody, no chronic rejection, acceptance of a 2nd graft, as well as donor-matched skin graft, and rejection of 3rd party allografts as a measure of immuno-competence. The other tolerance-inducing protocols discussed have not been fully evaluated in multiple strains, although a variation of the Sykes model has been used successfully in larger animals and nonhuman primates. However, concerns were raised by some participants about immuno-competence of the treated animals following the Sykes model, and/or the presence of chronic histo-pathologic changes in the graft in some situations following the Wood model. Based on this discussion, there is still a significant amount of basic science experimentation required before we have an ideal model of tolerance in rodents. It is only when such a model is established that we can perform a variety of rigorously controlled laboratory tests to determine which one(s) best predict or correlate with the tolerant state.

Despite the above deficiencies, the group next discussed how one would go about measuring tolerance in humans. It is obvious that certain experimental protocols, including placement of 2nd grafts or skin grafts, could not be performed in clinical trials and that other surrogate markers need to be established. It was further noted that long-term graft survival with normal graft function in the presence of minimal immunosuppression might suggest a clinically tolerant state but that more stringent immunologic criteria might help define truly tolerant individuals. Eventual, total discontinuation of immunosuppression with maintenance of normal organ function is the ultimate goal. The proposed tolerance assays would be used to evaluate the alloimmune repertoire in an effort to confirm tolerance and to define the presence of a potentially pathologic immune response prior to graft dysfunction.

A variety of assays capable of measuring immune reactivity were discussed with the caveat that there are no definitive data and only preliminary information about any of the proposed assays. Proliferative hypo-reactiveness to donor cells in a Mixed Lymphocyte Reaction (MLR), the lack of killing in Cytolytic T Lymphocyte (CTL) assays, the absence of donor-reactive recall Enzyme-Linked Immuno-SPOTs (ELISPOTs) for type 1 cytokines (i.e., IFNγ), and the absence of mRNAs for perforin/granzyme in graft biopsies, and the absence of alloantibodies were considered consistent with, but not diagnostic of, a tolerant state. In some situations, the presence of micro-chimerism was also considered by the group to be consistent with tolerance. An additional intriguing assay discussed by the group was the trans vivo Delayed-Type Hypersensitivity (DTH) assay. In this assay, control antigens and solubilized donor antigen are mixed with recipient cells and tested for their ability to mediate DTH in mice by simply measuring skin thickness. Recent studies have shown that Peripheral Blood Leukocytes (PBLs) from some tolerant patients can specifically suppress otherwise potent immune responses as measured by this assay, providing a readout that correlates well with tolerance. Whether or not this approach or any of the proposed assays will be useful for defining or identifying tolerance in the clinic remains to be determined in prospective trials.
The group consensus was that many of the proposed assays need to be evaluated in the context of animal models in which stringent criteria can be used to define tolerant versus nontolerant states. Until this is done, it will be difficult to interpret any results of studies performed in humans. Nonetheless, attempts at human tolerance induction are being performed in the clinical setting, and immune monitoring is therefore desirable. It seems that under these circumstances, the best approach to evaluate the usefulness of any of the proposed assays is to simultaneously study the same tolerant and nontolerant individuals (defined based on clinical criteria) using as many of the available assays as possible. In this way, it might be possible to correlate responsiveness, lack of responsiveness, or the presence of regulatory immunity with a particular clinical outcome. This is indeed the approach being championed by the NIH-funded Immune Tolerance Network, a multicenter program focusing on induction and measurement of tolerance in human disease. It is hoped that this effort, among many others, will provide us with better insight into the best ways to define and measure tolerance in the laboratory—and at the bedside.

References


The “cost” of transplantation has been, and remains to some extent, a matter of considerable controversy. Although debate concerning insurance coverage of specific organ transplant procedures has all but vanished, there is still the odd skirmish over the level of reimbursement that providers receive, and the financial obligations patients are forced to endure. Although the picture may not be as attractive as Marilyn Monroe, it is more handsome than Cary Grant.

But times remain both troubled and uncertain. Increasingly, amid a sea of red ink, transplant programs are running afoul of institutional efforts to either maintain or achieve financial solvency. Once considered to be the cornerstone of high technology, the foundation for research and innovation, a harbinger of success, and a marketer’s poster child, transplant programs are hitting the chopping block with about the same enthusiasm as a Perdue chicken headed to Popeye’s.

In anticipation of this inevitable state of affairs, I have, on many occasions, climbed atop a soapbox and pontificated at length about the economics of transplantation. Sobering thoughts have often been sandwiched between humorous sentences. However, most people preferred to chuckle than to knuckle, and a few got downright mad. As a result, despite my efforts to impart knowledge, I entertained, and absorbed the odd comment disparaging my ancestry. In the end, I must confess, people prefer to ignore what they don’t want to hear.

The level of institutional self-promotion associated with transplantation has often amused me. Unfortunately, like “New Coke” in 1985, great marketing concepts sometimes lack business sense. In health care, this is usually apparent when hospital boards call in management consultants and other dubious characters to “turn around” failing medi-

ical centers. Popeye’s “little helper” typically exercises a “one-size-fits-all” mentality—chop, chop, chop—and, as their critics point out, when it comes to economics, these folks are savages.

In my opinion, terminology is the root of the problem. Words typically get in the way of understanding. This is certainly true in any attempt to appreciate the nuances associated with the economics of transplantation. Although most people now realize there is a difference between a cost and a charge, when more detailed discussion ensues, my level of amusement is soon on par with a Sanford and Son or Monty Python rerun.

Over the past decade, hospitals and health care systems have periodically grappled with the problem of controlling “costs.” In an effort to do so, most hospitals and health systems have managed to cobble together accounting systems with the versatility of a four-function calculator. This is not surprising. In more lucrative times, it wasn’t even necessary to understand “production” or actual costs since reimbursement typically equaled or, at the very least, approximated billed charges. Frankly, there was more gravy in the system than the entire chain of Cracker Barrel restaurants has served over the course of its corporate life. In this era, cost containment meant increasing charges to enhance margins, without the faintest understanding of true costs.

“Expense management” is a concept that has now achieved buzzword status. It has captured the imagination of the proverbial bean counters. A new fetish has even emerged—a clinically morbid fascination with the bottom line. To relieve anxiety, there has been a proliferation of “decision support systems.” These mindless contrivances enable accountants and “financial analysts,” with varying degrees of expertise, to allocate costs, set charges, project reimbursement, and speculate about margins. In short, for clinical “product lines,” decision support systems abstractly identify the “winners” and the “losers”—those services that make and lose money. Transplantation is often considered suspect because it has a tendency to generate a lot of revenue, but little, if any, margin.

In reality, decision support systems consist of nothing more than computer software—if you will, metaphorically like a cowboy with a big hat and no cattle. The software manipulates
extant data according to various accounting principles—principles that are often modified to meet the needs of a particular external “client,” or internal “customer.” This feature is necessary since few hospitals and healthcare systems follow precisely the same rules in deriving their costs, allocating their expenses, setting their fees, and determining their charges. Ultimately, a diversity of accounting principles has its benefits when accountants fail to adequately explain results.

Unlike science, accounting seems to place little value on replication. Therefore, different accountants and financial analysts essentially use the same system to provide widely discrepant results and divergent conclusions, all of which depend on the desired answer. (This isn’t hypothesis testing, it is belief confirming.) Thus, when a transplant program is about to face the chopping block, there are usually endless analyses by different persons who offer inconsistent interpretations of the same data, all in a predictable direction.

Now that decision support systems have seemingly metastasized throughout the entire healthcare system, a whole host of folks have become expert financial analysts. Many now bear the in vogue credential—a master’s in business administration (M.B.A.). These weekend warriors, unlike the U.S. National Guard, have spent a bit of their rest and relaxation time earning some administrative “stripes,” thus enabling them to function as “physician managers” or, worse yet, managers of physicians. When approached by one of these painful characters, I reach for the old standby—Preparation H—to relieve my hemorrhoidal symptoms.

I believe we have finally reached a point where hospital officials are beginning to reconsider the market value of what they now confess are “loss leaders”—services that have marketing appeal, but for which there is little expectation of a favorable margin. And, as my foregoing remarks imply, while many arguments have been used to justify transplant centers, there remain some very outspoken critics of the alleged “proliferation” of centers. In addition, analogies with the space program have, thus far, proven to be wide of the mark. As a result, perhaps the chicken has come home to roost as transplant volumes have moderated in response to donor organ constraints, and transplant iatrogenesis has become increasingly expensive, in both medical and human terms.

As currently practiced, transplantation is anything but a growth industry. Based on existing technology, it is a service that has reached its market potential. And, as financing has become a more pressing and widespread concern for most segments of the healthcare industry, transplantation programs are falling victim to both the success and the inherent limitations of their own technological underpinnings.

Admittedly, it is no fun being fiscally responsible when the goal is to save lives. Ignoring evidence to the contrary, physicians and surgeons continue to erroneously believe that life has no price. Thus, national policy dictates that we first transplant those patients who will have the poorest outcome at the greatest expense. This practice ultimately shortens the life of patients, compromises any benefits they may enjoy while living, and will almost certainly undermine the survival of transplant centers as well. Ethical arguments to the contrary are as bogus as the proponents of cost-ineffective health care.

There are many ways to assess the economic toll of transplantation. It can be examined from the perspective of the individual procedure, evaluated in terms of aggregate expenditures, or, in the case of a health plan, the impact can be computed on a per-member-per-month basis. Each perspective yields a different picture. Individually, transplant procedures are very expensive. In the aggregate, expenditures associated with transplantation are unremarkable. And, from the perspective of a large health plan, the expense of transplantation is almost trivial.

I have always maintained that a paucity of donor organs has favorably limited the economic implications of transplantation. If every person who might benefit from a transplant received one, the economic burden would be much greater, and the impact would be felt at every level, regardless of perspective or participant. In short, considering the possibilities, the debate concerning resource allocation could easily be far more vigorous than it has been.

I have also insisted that, in the United States, managed care has done more to moderate the economic costs of transplantation, both individually and in the aggregate, than any drug, medical, or surgical innovation of the past 20 years. It is foolhardy to think otherwise. When insurers moved from generous payments based on discounted billed charges to case rates, transplantation actually became a far less expensive intervention. No immunosuppressive drug has even come close to producing a similar effect.

However, depending on one’s perspective, the situation I describe here has been both good and bad. In reality, in addition to capping prices, managed care has had another effect. There has been a reallocation of increasingly scarce resources, much to the detriment of transplant centers. In effect, margins and profits within the system have simply been reallocated among the participating parties. Providers are making less money than they were and writing off more; insurers are less generous in their reimbursement and limiting the size of their annual premium increases; patients enjoy fewer benefits and are paying more out of pocket; pharmaceutical companies are experiencing record profits and are enduring serious criticism for doing so. In the end, transplant centers have been the big losers.
Clearly, if it is not obvious by now, what we have here is the proverbial shell game where no one seems intelligent enough to grasp and convey the big picture. I have tried to do so, but I am convinced that clinical judgment often stands in the way of prudent healthcare policy. The problem of seeing the forest for the trees is unavoidable.

The picture I have painted is dismal, but I firmly believe that institutional pressures on transplant programs to cover their costs and to turn a profit will persist. Programs can no longer be sustained at or below cost. Intra-institutional “charity programs” or “loss leaders,” where there is little or no expectation of a payoff relative to investment, are less appealing when the technology has become established and the service routine.

“Cutting edge” technology has a short shelf life, and in the case of transplantation, there has been a relentless pursuit of new “gimmicks” that will enable transplant centers to distinguish themselves among their competitors. Recent examples include living-related lung transplantation, living donor liver transplantation, and laparoscopic nephrectomy. In reality, these are not new technologies at all. They are merely variations on old themes, and not particularly persuasive ones at that.

Thus, when the roosters start crowing, the decision support systems begin churning, and the hospital officials call in the management consultants, transplant programs will experience firsthand the lesson I have preached—life does have a price, and some clinical programs intended to ensure human longevity are more valuable than others. Consequently, most transplant centers would do well to build their case now to minimize the discomfort associated with a stretched neck and to avoid the disquieting sound of yet another devastating hatchet job—chop, chop, chop.
Index to Graft
Volume 4

Issue 1, (January/February, 2001), pp. 1-88.
Issue 2, (March, 2001), pp. 89-150.
Issue 6, (September, 2001), pp. 385-468.
Issue 7, (October/November, 2001), pp. 469-520.

Author Index

Abcarian, H., 526.
Abecassis, M., 398, 474.
Abouna, G.M., 120.
Abtahi, P., 266.
Alwayn, I.P.J., 23, 50.
Anaizi, N., 232.
Awwad, M., 23, 36.
Ayares, D., 80.
Azimzadeh, A., 10.
Baden, L., 276.
Barreau, N., 135.
Barth, R.N., 105.
Basker, M., 23.
Bedard, E., 96.
Benedetti, E., 526.
Berney, T., 535.
Bishop, D.K., 508.
Blancho, G., 135.
Bloom, E.T., 160.
Bollinger, R.R., 416.
Bracy, J.L., 102.
Buell, J.F., 205.
Buhler, L., 36, 99, 535.
Cao, S., 202.
Caulfield, A., 535.
Cassileth, B.R., 146.
Chen, R., 355.
Chen, Z.-c., 32.
Cicalese, L., 526.
Clark, D.A., 338.
Clark-Borre, L., 391, 392.
Colman, A., 80.
Colvin, R.B., 44.
Cooper, D.K.C., 6, 23, 36, 94, 137.
Cosimi, A.B., 146.
Cowan, P.J., 47, 76, 78.
Cozzi, E., 66.
Daar, A.S., 164.
Daha, M.R., 188, 220.
Dahlberg, R., 449.
Dai, Y., 80.
Dalmasso, A.P., 53.
d’Apice, A.J.F., 47, 76, 78.
Davis, R.S., 300.
de Haij, S., 188, 220.
Donian, R., 491.
Dorling, A., 72.
Edge, A.S.B., 118.
Erulk, E., 300.
Evans, R.W., 154, 467, 574.
Farivar, R.S., 355.
Fischer, S., 481.
Forest, S., 369.
Friend, P., 418.
Friend, P.J., 66, 111.
Galili, U., 32.
Gasser, M., 346.
Gianello, P., 18.
Gocki, H., 76, 78.
Goddard, M., 66.
Gollackson, B., 23, 137.
Goodman, D.J., 47.
Gorczynski, R.M., 338.
Grassl, J.S., 445.
Groth, C.G., 115.
Hammer, C., 108.
Hanaway, M.J., 205.
Heeger, P.S., 195, 571.
Heinrichs, D.F., 407.
Holmeyr, S., 369.
Howard, R.J., 424.
Huang, S., 326.
Iacomini, J., 102.
Inverardi, L., 209, 519.
Jacobs, C.L., 410.
Jamieson, I.R., 424.
Joyce, J., 452.
Kalayoglu, M., 205.
Katz, J., 276.
Khabbaz, G.R., 435.
Keshavjee, S., 491.
Kim, D.Y., 500.
King, S.R., 491.
Klintmalm, G.B., 421.
Kobayashi, T., 27.
Koffron, A., 474.
Korsgren, O., 115.
Koski, G., 146.
Lake, K.D., 427, 544.
Larson, T.S., 500.
Link, C.J., 32.
Lipori, P., 424.
Lorenzini, R., 68.
Lucey, M.R., 223.
Lundin, S., 150.
Macchiariini, P., 10.
Mager, J.C., 508.
Martinez, D.M., 180.
McEwan, R.N., 432.
McKenzie, I.F.C., 83.
Michaels, M.G., 129.
Moore, M., 80.
Morel, P., 535.
Morgan, B.P., 63.
Morgan, M.F., 40.
Mullon, C., 126.
Musat, A., 205.
Nicks, M.W., 290.
Oberholzer, J., 535.
Oh, S., 383.
Olivier, D.L., 435.
Oroz, C.G., 365, 369.
Ortiz, J., 202.
Patience, C., 133.
Paul, D.C., 188, 220.
Pieroni III, R.N., 10.
Pino-Chavez, G., 60, 66.
Platt, J., 8.
Podnos, Y.D., 202.
Rastellini, C., 526.
Remersnyder, J.P., 146.
Ribo, S.C., 50.
Rose, A.G., 14.
Rose, M.L., 57.
Rothblatt, M., 143.
"The Use of Ex Vivo Xenogeneic Whole Liver Perfusion as a Bridge to Liver Regeneration or Liver Transplantation" Abouna, 120.
"Transplant Center Marketing" Zavala, 412.
"Transplant Infrastructure" Thomson, 403.
"Transplanting Organs from Pigs Transgenic for a Single Human Complement Regulator Protein" Schmoockel, Cozzi, Dunning, Goddard, Pino-Chavez, Friend, Wallwork, and White, 66.
"Treating Acute Liver Failure with an Extracorporeal Liver-Assist Device" Mullon, 126.
"Treatment of Groin Lymphocele Following Liver Transplantation with Fibrin Glue" Podnos, Ortiz, Ji, Cao, and Imagawa, 202.
"Understanding and Achieving Accommodation" Dalmasso, 53.
"Understanding and Preventing the Coagulation Disorders Associated with Xenograft Rejection" Alwayn and Robson, 50.
"Understanding Cultural Perspectives on Clinical Xenotransplantation" Lundin, 150.
"Understanding Hyperacute Rejection of the Lung: Is This a Special Case?" Pierson III, Macchiarini, and Azimzadeh, 10.
"Understanding Public Attitudes to Clinical Xenotransplantation" Cassileth, Remensnyder, Koski, Surman, and Cosimi, 146.
"Understanding the Immune Protection Afforded by Endogenous Complement Regulatory Molecules" van den Berg and Morgan, 63.
"Understanding the Induced Antibody Response" Galili, Chen, Tanamura, Seregina, and Link, 52.
"Understanding the Mechanism of Acute Cellular Rejection" Rose, 57.
"Understanding the Mechanism of Hyperacute Rejection" Platt, 8.
"Understanding Xenophobia Induced by Economics" Evans, 154.
"Xenotransplantation and Endothelium" Farivar, Chen, and Adams, 355.
"Xenotransplantation—A Closer Look pt 1" Cooper, 6.
"Xenotransplantation: A Closer Look pt 2" Cooper, 94.
"Xenotransplanting Neural Cells" Edge, 118.
"Xenotransplanting Pancreatic Islets" Konggren, Wennberg, and Groth, 115.

Biotoon
"Expression and Function of CD40 on Various Cell Types," van Kooten, de Hijai, Paul, and Dah, 220.

Endpage/Mismatches
"Taking Aim: Reflections on Quality of Life Research—A Sinner's Plea for Salvation" Evans, 467.

"The Unkindest Cut: Where Are All the Transplant Programs Going?" Evans, 574.

Forum Articles: Complexity
"Engineering an Immune System" Forrest and Hofmeyr, 369.
"How Complexity Helps To Shape Alloimmunity" Oroz, 365.
"How Does the Immune System See To It That It Is Doing a Good Job?" Segel, 370.
"Immunity as a Swarm Function," Gross, 369.

Literature Reviews
"Cell Transplantation," Inverardi, 519.

Meeting Reports

Methods

Miscellaneous
"Pharmaceutical Clinical Trials—All Phases," 216.
"Pharmaceutical Clinical Trials—Phase Three," 212.

State of the Art