Presidential Address
Hans W. Sollinger, MD, PhD, 1996-1997

Ladies and Gentleman, Members, and Guests:

Before I begin to tell you the story of Ray Owen, a name possibly unknown to many young transplant surgeons, I would first like to apologize for what seems to be a rather self-promoting title of this lecture. This was not meant to be so, but I rather wanted to stimulate your curiosity about whether I would talk about UW Solution, CellCept, or the Green Bay Packers.

First of all, it is my great pleasure to thank the membership of this society for giving me the privilege to serve as your president during the past year.

My thanks also go to the members of my team in Wisconsin. They are my colleagues and friends, and make it fun for me to go to work every morning. In particular, my thanks go to the late Folkert O. Belzer, my former Chair and mentor. Last, but not least, to my family—Mary, Niki, and Muffy are here today—I thank them for their love and support.

Now, let's walk down the campus of the University of Wisconsin. At approximately this spot here 50 years ago, you would have found the Institute of Genetics, a building which housed the laboratories of such great investigators as Al J. Kohl and M. R. Irwin and, of course, Ray Owen.

This is the building in 1997, beautifully restored and an attractive centerpiece of the School of Agriculture.

This is the young Dr. Ray Owen, at the time he performed his seminal experiments at the University of Wisconsin, which I would like to discuss with you today, and whose consequences have been so far reaching. Bernard Amos expressed it eloquently just last year: "A younger generation may be quite unaware of the tremendous impact Ray's observation and deduction had on biology. The implications of acquired tolerance led to three different Nobel Prizes: Medawar-Burnet, Snell-Dausset-Benacaerraf, and Doherty-Zinkernagel."

Ray's comments at the time of his discovery were more characteristic of his very humble nature: "Several interesting problems in the fields of genetics, immunology, and development are suggested by these observations."

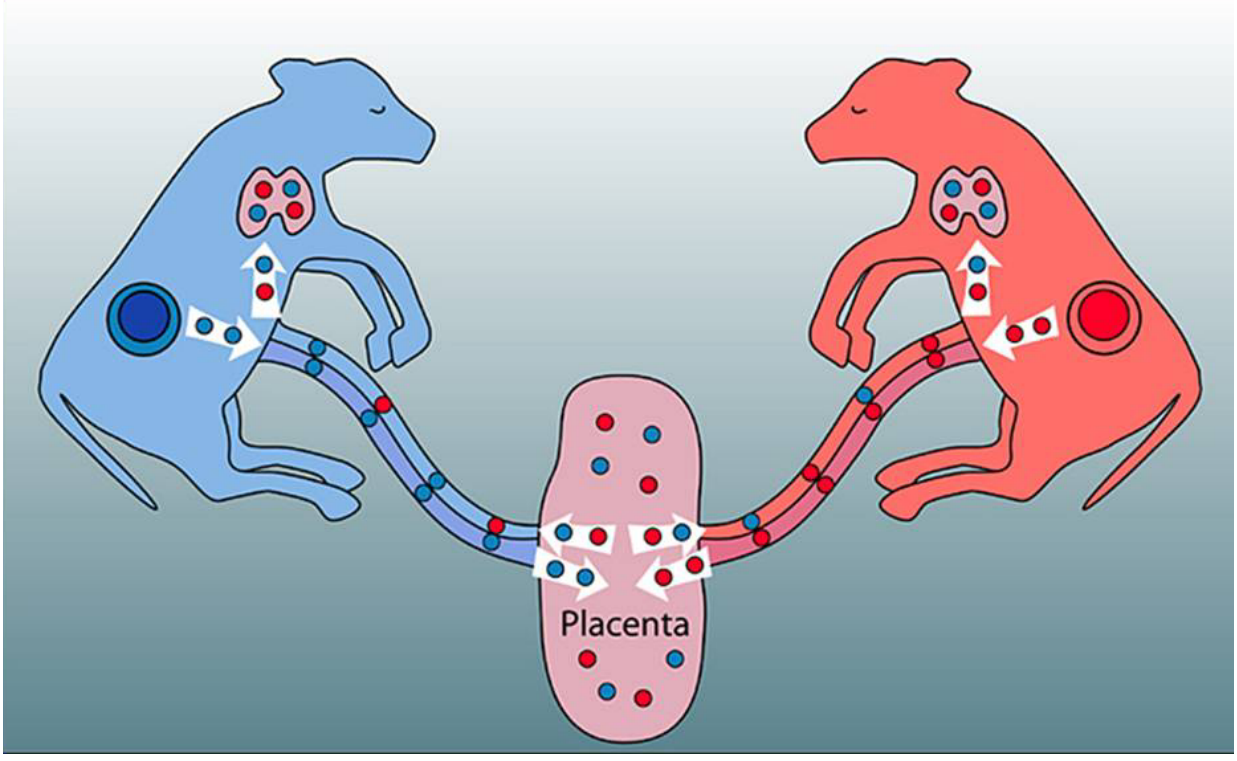
The very observation which stimulated Ray's interest in the discovery of red cell chimerism, however, is known by few, and I have to share this anecdote with you.

The events involved a Guernsey cow with twin calves. She had been properly mated to a Guernsey bull, but shortly afterward, a lustful Hereford escaping from a neighboring area got into the act. The color patterns of the calves showed clearly that the twins had different fathers.

The big surprise came with the blood group analysis. They had identical blood groups. This could not be explained by their being identical twins, for they were of different sexes, to say nothing of having different fathers.

The question arose, why should non-identical twins be identical for these blood groups, and how could a calf inherit blood groups from both fathers?

Ray, with his rural background, was familiar with the peculiar uterine anatomy of cattle, which facilitates cross-connections between the extraembryonic blood vessels, as demonstrated by Lilly as early as 1916. These anastomoses provide a ready opportunity for exchange of blood between the two embryos.



Further studies with cattle twins and their blood groups confirmed that non-identical cattle twins share red blood cells of the other twin, and these red blood cells remain in the animal's circulation for the rest of their lives. This observation resulted in the seminal paper in *Science* entitled "Immunogenetic Consequences of Vascular Anastomosis Between Bovine Twins."

What is not widely known is that Ray had initially submitted a much more detailed paper in which he extensively described the potential application of his observations for the field of immunology and transplantation. However, this paper was rejected and later reduced to the *Science* version.

The story moves now from Wisconsin to Australia, where McFarland Burnet and Frank Fenner were developing the concept of self and non-self in antigen recognition. In their 1949 book entitled *The Production of Antibodies*, they clearly recognize the significance of Ray Owen's observation. In their manuscript they write:

"There is interchange of embryonal cells, and these cells are capable of becoming established in the hematopoetic tissues of their co-twin hosts, and continue to provide a source of red blood cells distinct from those of the host, presumably throughout life."

From Australia we move to England, where Sir Peter Medawar, although having already written the classical paper on homograft rejection, was not aware of Owen's findings. In fact, it has been reported that in 1949, during a conversation with a Scottish veterinarian named Hugh Donald, who asked Medawar for a simple method to distinguish identical vs. non-identical calves, he replied, "Just exchange skin grafts between them, and if they get accepted, the animals are identical."

Here you can see a specimen of this surgical enterprise, and here, to the astonishment of their transplanters, these skin grafts were uniformly accepted. This was totally unexpected, as with an absolute statistical probability, only a few of these cattle could have been identical twins. The histology of the skin grafts was even more revealing.

As expected, a third-party skin allograft was rejected by a massive cellular infiltrate, while autografts, as expected, took perfectly well.

On a close look, however, while macroscopically skin grafts from non-identical twins were accepted, in contrast to the autografts, there was a mild infiltrate. Possibly, this is the first indication that an active interchange between the graft and the host takes place to maintain the status of graft acceptance.

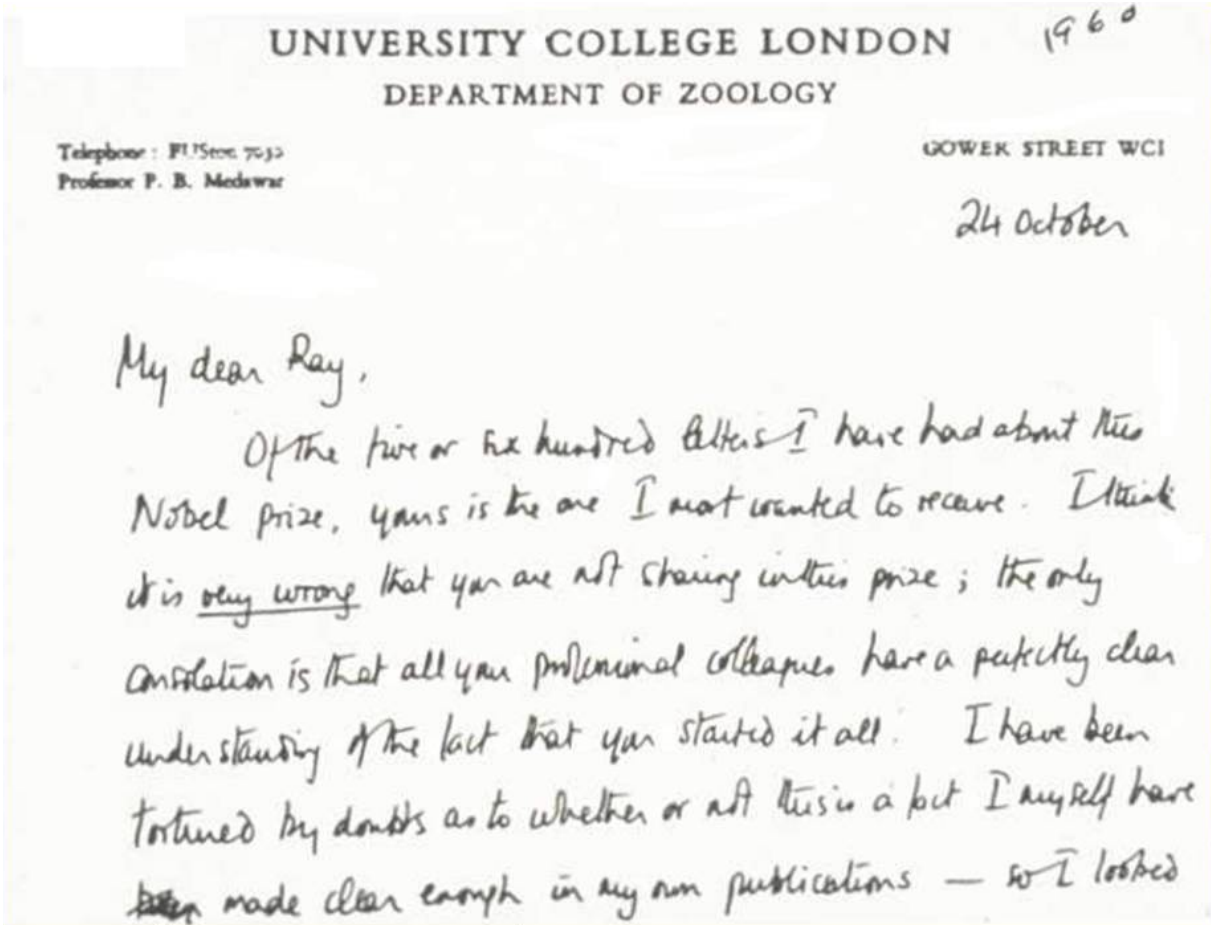
In 1951, when Medawar moved to London, his associate, Billingham, and student, Brent, went to work on their classical studies on the induction of tolerance in neonatal animals. Newborn mice or mice *in utero* were injected with donor cells and later grafted with the cell donor's skin graft.

These grafts were accepted for a prolonged period of time, and were the first demonstration of actively acquired tolerance of foreign cells.

The subtitle of the paper emphasizes the first demonstration of the induction of specific tolerance to murine skin allografts. This manuscript contributed greatly to Sir Peter Medawar's receiving the Nobel Prize in 1960. In retrospect, it was obvious how the Billingham-Brent-Medawar experiment was influenced by Owen's observation, but this was not recognized by many.

However, Peter Medawar, in his letter to Ray Owen, written shortly after receiving the Nobel Prize, demonstrates that the Nobel laureate knew otherwise. The letter reads:

"My dear Ray, Of the 500 letters I have had about the Nobel Prize, yours is the one I most wanted to receive. I think it is very wrong that you are not sharing in this prize. The only consolation is that all your professional colleagues have a perfectly clear understanding of the fact that you started it all…. Yours ever, Peter."



UNIVERSITY COLLEGE LONDON 1960

DEPARTMENT OF ZOOLOGY

Telephone: [illegible] Professor P. B. Medawar

GOWER STREET WC1

24 October

My dear Ray,

Of the five or six hundred letters I have had about the Nobel prize, yours is the one I most wanted to receive. I think it is very wrong that you are not sharing in the prize; the only consolation is that all your professional colleagues have a perfectly clear understanding of the fact that you started it all. I have been tortured by doubts as to whether or not this is a fact I myself have made clear enough in my own publications — so I looked

Medawar, at that time, did not seem to realize the implications which his experiment could have on clinical organ transplantation, and this anecdotal conversation has been reported on numerous occasions. Medical student Roy Calne: “Sir Peter, do you think your findings could have any implications for transplantation?” Medawar’s response: “Absolutely not.”

A precise account of the events which followed this seminal discovery is summarized in Leslie Brent’s recently published book, *A History of Transplantation Immunology*—a must-read for all involved in transplantation and transplantation biology. Many investigators have since contributed in attempts to induce tolerance on a clinical or preclinical level. I must apologize to all of those individuals who I will not be able to mention during the short course of this lecture.

One of the early pioneers utilizing antilymphocyte globulin and inoculation of bone marrow was former President Tony Monaco. Here, Tony is seen in conversation with Peter Medawar and Michael Woodruff.

The next giant who appeared on the scene was Dr. David Hume. As I was told, he called his young associates, Judy and Frank Thomas, into his office, and ordered them to initiate experiments which would ultimately lead to the long-term acceptance of allografts.

Unfortunately, Dave Hume died too early to enjoy the classical manuscript of Judy and Frank, published in 1987.

Frank and Judy and their collaborators could demonstrate that the combination of antilymphocyte globulin and donor-specific bone marrow infusion resulted in spectacularly extended graft survival in Rhesus monkeys, and even tolerance in some of these animals.

Walking through the poster session of the 11th International Congress of the Transplantation Society in Helsinki, Judy called me over to look at her poster. She pointed to a cluster-like aggregation of lymphocytes in these long-term accepted kidneys. I found the observation interesting; however, did not believe that any great deal of significance should be attributed to them.

Immunoperoxidase stains of the nodules with anti-donor sera obtained from Hans Ballner seemed to indicate that these nodules primarily consisted of donor type cells. However, as Judy recently indicated in a letter to me, staining techniques, as well as antibodies at that time, were crude, and it is possible that the quantity of donor cells was overestimated.

Nevertheless, they made the important observation that the presence of dense foci of CD8+ cells in the kidneys of long-term survivors was an unusual and fascinating finding of these studies.

Now, for a short while we seemed to have drifted away from Ray Owen's discovery. Nevertheless, there was one transplant surgeon—and possibly the only one—who had kept Owen's discovery in mind. Tom Starzl, as early as 1962 in the *Surgical Clinics of North America*, published this drawing of the Owen cattle twin experiment, demonstrating that he was very much aware of the significance of this experiment.

One of the crucial observations which Tom Starzl made came during his first Denver series of kidney transplants where a drug cocktail was used. He writes, "The fundamental observation was that something changed during the first weeks and months after successful kidney transplantation in the relation of the recipient to the graft. The pattern of recovery, in which the amount of drug treatment often became progressively less, was the strongest testimony that such a host-graft change had occurred at an early time, allowing the lifetime rehabilitation of such patients."

He noted that of the first 64 patients in the Colorado series, 16 survived for the next 25 years, with two eventually stopping all immunosuppression without rejection.

Starzl now drew a direct line between the Owen cattle experiments to Medawar, Billingham, and Brent, to the parabiosis experiments by Hasek and others, and arrived at a new concept entitled "The Two-Way Paradigm of Host-vs-Graft Reaction."

In a letter to me, Tom insisted that the credit for this discovery must be shared with Noriko Murase, Jake Demetris, and Mauricio Trucco. In his by now classical drawing, he postulates that a not-so-defenseless graft, or cells within this graft, in interchange with immunocompetent cells of the host, if the balance is right, and as indicated by persistent long-term chimerism, might result in long-term graft acceptance.

Clearly, this observation was hotly debated and often questioned by scientists and clinicians of the highest caliber. The observation that patients who are chimeric reject their grafts and that non-chimeric patients seemed to do perfectly well suggested that chimerism is just an epiphenomenon of successful immunosuppression.

It was therefore of interest when Will Burlingham, here shown with Ray Owen, was able to identify a young patient who had received a kidney from his mother many years ago and had stopped immunosuppression for several years. Will isolated donor-specific cells from the blood of

the recipient and demonstrated that addition of very few of these cells could reduce, or even abolish, donor-specific cytotoxicity. Thus, the functional capacity of donor-derived chimeric cells was first demonstrated.

Furthermore, biopsies obtained from this patient demonstrated lymphocyte clusters reminiscent of the ones the Thomases had shown us 19 years before. PCR analysis of these clusters was striking for a high concentration of TNF-α, a highly potent apoptotic cytokine. This observation clearly points to the significance of the graft itself as the important component in the induction of unresponsiveness. This observation might well resolve the controversy between those who believe peripheral chimerism is of importance and those who don't.

I would suggest that in some cases, these apoptosis-inducing cells are present and can be detected in the circulation, and in some cases, they produce their potent function within the graft without being detected in the circulation. In any event, the end result—graft acceptance with no or minimal immunosuppression—is the same.

Our task now is to develop strategies to intentionally design protocols which allow this beneficial interplay between graft and host responses to take place, and to create a truce between host and recipient. Two of my colleagues, Stuart Knechtle and Allan Kirk, have recently developed

protocols in MHC Class I and II mismatched Rhesus monkeys which have the potential to reach this goal. First, Stuart demonstrated that a short course of immunotoxin and donor lymphocyte resulted in long-term graft survival of kidney transplants in these animals. It is of interest that in Stuart's experiments, the administration of immunotoxin was followed by profound T cell depletion, lasting two to three weeks before recovery occurred.

In contrast, Allan Kirk, using the same experimental model, with the administration of two monoclonal antibodies, blocking costimulation to CD28 and CD40, achieved an equally promising result. In his experiments, however, no T cell depletion was seen. However, in both of the long-term surviving animals, again, typical lymphocyte clusters were prominent on biopsy.

In summary, these primate experiments, the work by Starzl and his group, earlier courageous clinical series by Barber, Diethelm, Monaco, and others, now open the stage to the investigation of preclinical and clinical trials, possibly supported with enthusiasm by the National Institutes of Health, to enter the Holy Grail of transplantation biology—long-term graft acceptance without immunosuppression.

Again, we owe great respect and gratitude to a very humble, brilliant scientist, who showed us the way more than 50 years ago. I am personally delighted that he did it in Wisconsin.

Thank you very much.