Rupert Billingham and the Role of Serendipity in the Discovery of Transplant Tolerance

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The Presidential Address gave me an opportunity to pay tribute to my teacher, Rupert Billingham, by recounting the early history of transplantation. Informal conversations with Billingham during the six years I spent in his department and during a recent visit to his retirement home on Martha's Vineyard provided me with some little known details of the story. Even ASTS members may not be familiar with all of the contributions that Billingham and others made (many of them largely by chance) to the discovery of tolerance for which Peter Medawar was awarded the 1960 Nobel Prize. Others who could have argued for a share of the prize include John Hunter, Frank Lillie, Ray Owen, Hugh Donald, Emile Holman, J. Barrett Brown, Charles Danforth and Francis Foster, Thomas Gibson, Bill Longmire, and Jack Cannon.

The story begins in the first decade of this century with the realization that even technically successful skin homografts uniformly fail. One of the first good descriptions of rejection is in a paper published in 1922 by Emile Holman, a 32-year-old surgical resident in Halsted's program at Johns Hopkins. He transplanted many small skin allografts to the leg of a 5-year-old boy who had lost most of the skin of his lower extremity in an accident. A month later, Holman performed more pinch grafts to obtain better coverage of the wound. The boy's mother was the donor of both sets of skin grafts. Holman noted that the "second set" of grafts were rejected more rapidly than the earlier ones, thus becoming the first one to describe the so-called second set reaction. Holman, who became chairman of surgery at Stanford, said later that one of the great missed opportunities of his life was a failure to follow up on this observation. Rediscovering the phenomenon, Medawar used it to prove that rejection was an immunological process.

In 1929, two Stanford veterinarians, Charles Danforth and Francis Foster,

observed that skin grafts exchanged between newborn chicks were usually accepted permanently. This suggestion that allograft rejection might be preventable was overlooked until 20 years later when the phenomenon was further explored by two UCLA surgeons, William Longmire and Jack Cannon. They found that skin allografts were rejected by chicks unless they were performed immediately after birth. Recognition that cellular or tissue allografts could survive if transplanted very early in life was crucial to the protocol used later by Medawar and his colleagues to induce tolerance.

Although he was aware of the work of Danforth, Foster, Longmire, and Cannon, it was only by chance that zoologist Peter Medawar became interested in transplantation. During World War II, Medawar was assigned to join plastic surgeon Thomas Gibson at the Royal Infirmary in Glasgow in studying homografts as a possible means of treating burned aviators. They reconfirmed that skin allografts always failed and also rediscovered the second set phenomenon previously described by Emile Holman. Importantly, they recognized that this proved that rejection was an immunological process.

In 1946, Rupert Billingham enters the story. Born in 1921, this grandson of a dairy farmer became Peter Medawar's first graduate student at Oxford. Interestingly, his work is not on the homograft reaction. Instead, he was assigned a project on the esoteric subject of pigment spread. The goal was to explain why the pigmented areas in spotted pigs gradually encroach on the white skin. To observe this phenomenon, Billingham and Medawar transplanted autografts of black skin to white areas on the same guinea pig. Their experiments were the subject of Bill's Ph.D. thesis and first eight publications.

Soon after this, Medawar accepted the chair of zoology at the University of Birmingham. Influenced by the knowledge that Medawar's technician Jean Morpeth had accepted a job at Birmingham, Billingham sought a position in his department where he continued to work on pigment spread.

In 1949, serendipity assumes crucial importance in the story. Medawar had a casual conversation at a cocktail party with a British veterinarian, Hugh Donald, whose research was on the importance of heredity vs. environment on animal behavior. As a model for his studies, he was using twin cattle, but was handicapped since he could not distinguish identical from fraternal twins with certainty. Medawar advised him that this should be no problem. Exchanged skin grafts should be accepted only by identical twins.

When Donald asked him to perform the skin grafting experiments, Medawar was reluctant on two counts: (1) Donald's cows were kept on a farm two hours from Birmingham and (2) Medawar had no experience handling large animals and was uncomfortable with the prospect. Therefore, he enlisted the aid of his junior colleague, Billingham, who was not only expert in skin grafting but as the grandson of a dairy farmer was not afraid of cows. Billingham and Medawar set out on this adventure with little enthusiasm. Although they had minimal scientific interest in the outcome of the skin grafting which they felt was predictable, Billingham, Medawar, and usually Jean Morpeth made many long trips to Cold Norton Farm to perform and examine the skin grafts.

Thrown together as collaborators on this strange project, Billingham and Morpeth were becoming progressively better friends, but the scientific aspects of the project were not going well. After studying 25 twin pairs, they found that 86% of exchanged skin grafts were accepted for greater than 100 days, a surprising result since most cattle twins are fraternal. They discussed this unexpected success of grafts exchanged between fraternal twins with Hugh Donald, who suggested that they read a paper published four years earlier in *Science* by Ray Owen. When they did so, the significance of their results suddenly became clear.

To place the cattle twin chapter of the story of tolerance in proper context, it is necessary to go back in time over 200 years. In 1779, English surgeon John Hunter provided the first anatomical description of the freemartin, a term used for the generally sterile female of a pair of cattle twins of unlike sex. Hunter dissected freemartins, finding that they had masculinized sex organs. That Hunter was unable to explain this curious phenomenon is ironic because he was an expert on the circulation of the placenta.

The next important link in the story was not provided until 1916 when Frank Lillie, an embryologist at the University of Chicago, was sent several specimens of unborn cattle twins. He found that chorions of the twins' placentas were fused, causing a common intrauterine circulation that allowed blood to be exchanged freely between the twins. Like John Hunter, Lillie also found that when cattle twins were of unlike sex, the gonads of the female were usually rudimentary. He reasoned that mole hormones circulating through the female embryo inhibited the development of its reproductive organs.

Three decades later, in 1945, Ray Owen, a 39-year-old assistant professor of genetics and zoology at the University of Wisconsin, wrote the next chapter. In studying the red blood cell types of cattle, Owen found that fraternal twins frequently had a mixture of two red blood cell types. Recalling Lillie's finding of placental fusion of bovine twin embryos, Owen concluded that not only hormones but also cellular elements of the blood must be exchanged in utero by twin cattle. He realized that persistence of red blood cell chimerism in adulthood must depend on intrauterine transfer not only of short-lived red blood cell but also of stem cells that would perpetuate them.

Six years after the publication of Owen's paper, Billingham and Medawar read it with fascination and were suddenly able to interpret the outcome of their cattle skin graft experiment. They realized that, like the freemartins studied by Hunter, Lillie, and Owen before them, their twins must have exchanged both cellular and hormonal components of blood in utero. As Owen had shown, their cellular chimerism persisted in adulthood. They reasoned that the stem cells exchanged would be not only those for red blood cells but also for white blood cells, and that since the latter were known to express transplantation antigens, these were probably responsible for skin graft tolerance. They also realized at once with considerable excitement that they could probably reproduce the phenomenon in other species.

In 1951, Billingham and Medawar moved to University College, London, where Medawar became chairman of zoology. It turned out to be a happy move for them both. Billingham and Morpeth were soon married. Medawar said "Thank God we've

left those cows behind." There, Billingham, Medawar, and graduate student Leslie Brent attempted to induce chimerism and tolerance in mice, a task which proved quite difficult. Initially they used a laparotomy to expose and deliver cellular inocula to fetuses, an operation that caused almost 100% mortality. Not until almost a year later did they hit upon the technique that allowed the first success. After making a skin incision, they could visualize the uterus through the intact but semitransparent layers of abdominal muscle and inoculate the intrauterine fetuses with donor strain cells. In retrospect, they were quite lucky to have achieved any successes. By chance, the inbred mouse strains they chose for the experiments were CBA and A, virtually the only H-2 incompatible strain combination available to them in which neither severe graft-versus-host disease nor incompatibility of skin specific antigens would cause death or rejection of the graft.

In adulthood, survivors of the intrauterine inocula were grafted with skin from the donor strains. Although the results were somewhat inconsistent, prolongation of skin graft survival occurred in 40% to 50% of the recipients. A manuscript was quickly prepared and submitted to *Nature*. But while it was being reviewed, Billingham, Medawar, and Brent were horrified to discover that, due to a mixup in their animal colony, some of their recipients were F_1's, which would accept the parental strain allografts on a genetic basis alone. They briefly considered withdrawing the manuscript but fortunately were able to conduct more grafting experiments in time to be reassured that their conclusions were valid.

Their brief paper in 1953 attracted the world's attention. Like the cattle work, it demonstrated that allograft rejection was not inevitable. They had shown that successful transplants could be achieved by a fairly simple protocol. Medawar said that its significance was moral rather than practical.

In 1960, Medawar was awarded the Nobel Prize, largely on the strength of the tolerance experiments. The prize was shared not with Billingham but with McFarlin Burnet, an Australian who had theorized on the mechanism of tolerance. Although Billingham has never acknowledged it, others have frequently asserted that omitting him was unjust. His role was crucial both in the tolerance work in mice and in the earlier cattle work, which was its genesis. To indicate his own recognition of Billingham's contributions, Medawar divided his prize money with Billingham (and Brent). Billingham used his share for a downpayment on a house in suburban Philadelphia.

In 1957, Billingham accepted an offer to head a transplantation research section at the Wistar Institute in Philadelphia. His first recruit, Willys Silvers, shared his interest in pigment spread. They collaborated on experiments on this phenomenon. Somewhat to Billingham's discomfort, they eventually discredited the infectious concept of pigment spread put forth in the early work with Medawar. Instead, they established that pigmented cells actually migrate from black skin grafts into the adjacent white skin of the recipient.

Throughout the remainder of his career, Billingham continued to explore the complex phenomenon of rejection by simple methods such as skin grafting. He said the ingenious experimental designs he used were in the Medawar style. But those of us who worked under him believed the approach to be Billingham's own hallmark.

Despite the many original and important publications generated by this work, neither Billingham nor Medawar was ever again to equal the spectacular success of the tolerance work. But perhaps no other experiment in transplantation has ever approached its impact.

Nevertheless, during his Philadelphia period, Billingham's ideas led to numerous and important findings by his group. Using the classic Billingham model of neonatal tolerance, his graduate student David Steinmuller demonstrated that passenger leukocytes migrating from skin allografts are in themselves sufficient to sensitize their hosts. Along with Wayne Streilein, Billingham pioneered tissue typing with a now-forgotten method called the irradiated hamster test. Through another graduate student, Darcy Wilson, he encouraged seminal work in the mixed lymphocyte culture test. With Will Silvers and with me, he studied privileged sites, such as the hamster's cheek patch. I was lucky enough to collaborate with him in devising an artificial privileged site, which confirmed the crucial importance of the lymphatic circulation in skin allograft rejection. Billingham's 1964 publication in the *New England Journal of Medicine* stimulated interest in immunological aspects of the maternal fetal relationship, a topic largely overlooked until then. Later, the American Society of Reproductive Biology, in electing him to its presidency, referred to him as "the father of reproductive biology." Also during this period, he helped me initiate the human kidney transplant program at the Hospital of the University of Pennsylvania.

In 1971, Billingham accepted an offer to chair the Department of Cell Biology at Southwestern University in Dallas. I feared that his move might end my scientific career, but the background he had provided allowed me to continue the study of privileged sites. The genesis of my group's subsequent work on pancreatic islets was the idea that these cellular grafts might be transplanted to privileged sites. In Dallas, with Alan Beer, Billingham continued his experiments on the maternal fetal relationship. Although they did not fully succeed in explaining the riddle of nature's uniquely successful allograft, their work was highly influential in the development of that entire field.

Ironically, like Peter Medawar's, Billingham's career was eventually cut short by a disabling neurological illness, Parkinson's disease. Nevertheless, his retirement has been gratifying in many respects. Honors have continued to flow his way, such as the honorary DSC from the University of Pennsylvania in 1993.

Medawar said that the impact of the discovery of tolerance was predominantly moral. But time has proven him wrong in believing it would never have practical importance. In fact, tolerance and chimerism seem to be increasingly important, as modern transplant surgeons—following the lead of Billingham, Brent, and Medawar—attempt to mimic nature's experiment, the freemartin. Protocols may eventually allow successful human allografts without immunosuppression. As evidence of the continuing influence of this work, I cite the 12 papers on the 1993 ASTS scientific program dealing with either tolerance or chimerism. Of particular note are the paper by Monaco's group, which for many years has studied the effects of chimerism on tolerance in mice; the attempts by Diethelm and Barber to induce toler-

ance to human kidney allografts with donor bone marrow; and Susan Ildstad's use of mixed donor-recipient lymphoid cell chimerism.

The Philadelphia group's use of the intrathymic tolerance model is a derivative of Billingham's work in two respects. First, intrathymic inoculation of islet cells into the thymus might never have been pursued if the prolonged survival of intrathymic islet allografts had not interested members of my group, brought up on stories of Billingham's fascination with the obscure phenomenon of immunological privileged sites. Second, in rereading the original tolerance papers, we were intrigued by the failure to induce tolerance with nonlymphoid cell inocula, such as kidney or testicle. We reasoned that success of lymphoid cells might depend on their unique capability to home to the thymus, but that kidney or islet cells would be equally effective if they could reach the thymus by purposeful implantation.

Finally, Tom Starzl's recent recognition of the emigration of passenger cells from solid organ transplants and their persistence in successful transplant recipients appears to indicate that these fortunate individuals are often tolerant. That some of them can even discontinue immunosuppressive drugs provides clinical confirmation of the importance of chimerism—a state first recognized in cattle twins and then induced experimentally by Billingham and Brent and Medawar.