Medawar Redux – An Overview on the Use of Farm Animal Models to Elucidate Principles of Reproductive Immunology

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Introduction

Reproductive immunology was born in the barnyard. Indeed, the seminal experiments that led to two of the major concepts underpinning reproductive immunology were conducted using the bovine as a model. Peter Medawar, the scientist who introduced the concept of the fetal allograft, formed his initial ideas regarding immunologic tolerance (from which grew the concept of the fetal allograft) while reading about and studying dizygotic twins in cattle. The importance of hormonal regulation for immune function in the reproductive tract, and the resultant consequences for resistance to venereal and periparturient infectious disease, was first identified by Lionel Rowson while working on developing methods for embryo transfer in cattle.

This volume of the American Journal of Reproductive Immunology is composed of review articles that highlight the continued relevance of farm animals as models for research in mammalian biology. As shown through these reviews, farm animals are providing important insights into the nature of the conceptus–maternal immunologic relationship (Noronha, Ott), hormonal regulation of uterine function (Padua), host defense mechanisms in the reproductive tract (Entrican, Hansen), role of endogenous retroviruses in placentation (Spencer) and involvement of the immune system in function of the corpus luteum (Pate). The purpose of this short introduction is to place the farm animal research model in a historical and evolutionary context.

A Little History

The story of the foundation of reproductive immunology illustrates the utility of using farm animals as models for studying mammalian biology. More
importantly, it teaches the importance of keen observation in biological research followed by the pursuit of the question Why?

The Fetal Allograft

The father of reproductive immunology is Sir Peter Brian Medawar (Fig. 1), whose paper describing the paradox of the fetal allograft\(^1\), whereby an immunologically distinct organism can develop within an immunologically competent host, gave birth to the still-vibrant field of pregnancy immunology. Medawar’s insights regarding the immunologic problems posed by vivaparity did not develop because of a long-term interest in the biology of pregnancy. Rather, he developed his concepts about the fetal allograft because of his work on immunologic tolerance for which he eventually shared the Nobel Prize with Frank Macfarlane Burnet in 1960. A key observation of Medawar’s research was that immunologic tolerance could be induced by antigen exposure in fetal life so that adults are tolerant of tissues expressing histocompatibility antigens that they were exposed to while fetuses.\(^2,3\)

The idea that immunologic tolerance develops in the fetus was first shown by the immunogeneticist Ray Owen of the University of Wisconsin (Fig. 1). A local farmer brought to the attention of the university a case of superfecundation where twin calves (in this case, of different sex) were sired by two different bulls. In cattle, vascular anastomoses form between placental blood vessels of twins. Based on the presence of blood antigens that the calves could not have inherited genetically, Owen concluded that the calves had exchanged cells during fetal life and that descendants of these cells persisted in postnatal life.\(^4\) Survival of the cell lineages in genetically foreign animals must have been dependent on immunologic tolerance.

Owen’s report stimulated Medawar to demonstrate immunologic tolerance experimentally. As Medawar states in his Nobel Lecture,\(^5\)

In 1945, R.D. Owen made the remarkable discovery that most twin cattle are born with a stable mixture of each other’s red cells; it followed, then, that the twin cattle must have exchanged red-cell precursors and not merely red cells in their mutual transfusion before birth. This is the first example of the phenomenon we came to call immunological tolerance...A few years later R.E. Billingham and I, with the help of three members of the scientific

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Fig. 1 Peter Medawar (top right), Ray Owen (bottom right) and an excerpt of a letter from Medawar to Owen, October 24, 1960. The image of Medawar is from Wikipedia Commons, and the other images are from the University of Wisconsin, Dept. of Genetics and are produced with permission.
staff of the Agricultural Research Council, showed that most dizygotic cattle twins would accept skin grafts from each other, and that this mutual tolerance was specific.

The results of these experiments were published by Medawar and colleagues in 1951 and then similar experiments to demonstrate immunologic tolerance in fetal mice were published in 1953.

As indicated from the excerpt from his Nobel lecture cited previously, Medawar acknowledged the intellectual connection with Owen’s work. In a letter to Owen in 1960, a portion of which is reproduced in Fig. 1, Medawar wrote:

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 this 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 publications.

Owen himself does not feel that his contributions were unappreciated. In a recent email communication, Owen stated that ‘I’ve never felt like I deserved or wanted a share in the Prize. Good thought on Medawar’s part, but I’d rather his note went without my formal approval’.

**Hormonal Regulation of Uterine Immune Function**

The problem of the fetus being an allograft only exists because the uterus is not an immunologically privileged site. Tissue allografts placed with the uterine lumen are readily rejected. The immune system surveils the reproductive tract not to inhibit establishment of foreign allografts but instead to prevent infectious disease in the reproductive tract. Proper functioning of the immune system is important for the prevention of infections caused by mating, parturition or clinical procedures.

One of the major regulators of immune function in the reproductive tract is the endocrine system. Of the myriad hormones that can affect uterine immune function, none has more profound effects than progesterone, the main hormone of pregnancy, which can block allograft and xenograft rejection and which also increases the likelihood that infectious disease follows the introduction of microorganisms into the reproductive tract.

The concept that progesterone can regulate uterine defense mechanisms is one that was developed using the cow as a model by Lionel Edward Aston Rowson, F.R.S. (or Tim as he was known) and colleagues of the Agricultural Research Council in Cambridge, England (Fig. 2). Like Medawar, Rowson’s immediate interest was not in reproductive immunology. His group was one of several working to develop procedures for embryo transfer. The first live calf born from embryo transfer was produced by Elwyn Willlet and colleagues at the American Foundation for the Study of Genetics in Madison, Wisconsin in 1950.

In their efforts to achieve successful embryo transfer, Rowson’s group attempted to transfer embryos non-surgically through the cervix, a procedure that would not become common until the 1970s, in large part because of Rowson’s efforts. Early efforts with transcervical transfer at Wisconsin and Cambridge were impeded by a high incidence of uterine infections in embryo transfer recipients. Faced with this difficulty, Rowson speculated that progesterone was
involved because transfers were performed during the luteal phase of the estrous cycle when concentrations of the hormone were high. This hypothesis resulted in a series of experiments described in a paper in 1953\textsuperscript{15} that provided experimental evidence that progesterone was, in fact, inhibitory to uterine anti-bacterial defense.

One key experiment was to ovariectomize cows and assign them to no treatment, stilbesterol (an estrogen), or stilbesterol followed by progesterone. Cows were inseminated with semen contaminated with bacteria \textit{[Arcanobacterium pyogenes (previously Corynebacterium pyogenes)] and occasionally other organisms} and the uterus examined for infection after slaughter 2 days later. Of the four untreated cows, three had sterile uteri at slaughter and one had only a few colonies of \textit{A. pyogenes} in one uterine horn only. The uteri of both cows treated with stilbesterol were also sterile. However, the uteri of all three cows treated with progesterone were filled with pus and large number of neutrophils, and large numbers of \textit{A. pyogenes} were present. Thus was obtained the first evidence that progesterone can modify the course of immune responses against microorganisms.

A Little Evolutionary Biology

When choosing an animal model for research, many considerations are made, including accessibility of animals and reagents, ease of handling, cost, knowledge of the animal’s biology and husbandry, the degree of acceptance of the animal as a model by the scientific community, and whether the animal is amenable to manipulation (for example, performing homologous recombination experiments). The most widely used animal model, the laboratory mouse, has desirable characteristics with respect to all of these considerations. Another important consideration is whether principles gleaned from one species are broadly applicable to other species. It is especially desirable that research be relevant to humans because of the paramount importance of research directed toward improving human health.

The concepts of immunologic tolerance and the immunosuppressive actions of progesterone first examined by Medawar and Rowson using cattle have since been shown to have general relevance for mammalian biology including that of humans. Given mammalian evolution, one could, in fact, predict that the biology of common farm animals would often be more similar to that of humans than is the case for mice. Even though the common ancestor of farm animals, such as cattle and sheep (Cetartiodactyls), pigs (Suidae) and horses (Perrisodactyls) diverged from humans before the common ancestor of humans and rodents, important features of the bovine genome are more similar to the human genome than is the murine genome. Rodents have experienced a high rate of evolutionary change. Mice have experienced twice the number of synonymous nucleotide mutations as humans since their divergence and 1.3 times the number of non-synonymous mutations.\textsuperscript{16} As a result, the amino acid sequence of most proteins is more conserved between cattle and humans than between mice and humans, and the number of unique orthologous groups is greater for rodents than for several other mammalian species (Fig. 3).\textsuperscript{17} In addition, chromosomal organization is more similar between cattle and humans than between humans and mice.\textsuperscript{17}

Many of the segmental duplications in the bovine genome involved immune-response genes and placental genes.\textsuperscript{17} Indeed, evolution of new genes for the control of placental function is a more general phenomenon. As a result, many genes overexpressed in the placenta or decidua arose recently in evolution so that orthologs do not exist in any but closely related species (Fig. 4).\textsuperscript{18} One example is the chorionic gonadotropin $\beta$ gene, which arose by gene duplication in primates about 34–50 million years ago so that prosimians and tarsiers, which diverged from anthropoid primates, do not possess a chorionic gonadotropin $\beta$ gene.\textsuperscript{19} A separate chorionic gonadotropin $\beta$ gene arose independently in equid species. A second example is the interferon-$\gamma$ gene, which arose in ruminants as a gene duplication of interferon-$\alpha$ about 36 million years ago so that the gene is limited to ruminants.\textsuperscript{20} The recent evolution of so many genes involved in placental function means that an understanding of key aspects of pregnancy biology in any species will sometimes require study of that species or a closely related one.

Conclusions – Reassessing the 98% Solution

Biomedical animal research is almost wholly a murine affair. Of the grants using rodent or domestic animal models funded by NIH from 2002 to 2006, 98% used rodents and, in most of these cases, mice.\textsuperscript{21} In 2006, there were 2262 grants funded for research using rodent models and 37 for grants
funded using farm animal models. Undoubtedly, the laboratory mouse has proven to be an invaluable model for biological research and most of what we know today about mammalian biology is derived from research carried out with *Mus musculus*. Nonetheless, to reject other animal models is to ignore the need to address evolutionary divergence among mammals by studying biology across an array of genotypes. Moreover, the opportunity to exploit unique biological models or intriguing insights can be squandered. Clarity about the nature of immunologic tolerance was developed because Owen and Medawar capitalized on the unique properties of the placental vasculature of twin calves. Rowson’s frustrations with uterine infections in embryo transfer recipients gave impetus to his fruitful studies that established progesterone as a key hormone regulating uterine immunity. The papers in this special issue of the *American Journal of Reproductive Immunology* highlight additional examples whereby farm animals are being used to develop concepts pertinent to a wide range of mammalian species.

Domestic farm animals are not the only mammalian species that can make useful research models, of course, but they offer advantages of availability, ease of handling, cost, and a well-described biology and husbandry. When Medawar was struck with the idea of using the calf in his research, he turned to colleagues at the Animal Breeding and Genetics Research Organization in Edinburgh. Today, unfortunately, the infrastructure for conducting farm animal research is eroding.\(^{21,22}\) For example, the number of scientist years working in animal production or protection in the United States declined 22% from 1985 to 2006 and doctorates awarded in the animal sciences in the United States declined by 30% from 1985 to 2004. An increase in investments in basic research using farm animals will have a positive impact not only on agricultural productivity but on understanding mammalian biology and enhancing human health.
Acknowledgments

During the initial preparation for this paper, I was fortunate enough to attend the celebrations surrounding the 100th Anniversary of the Dept. of Genetics at the University of Wisconsin. In the course of the event, I heard details of the contributions of Ray Owen to the idea of immunologic tolerance that I was unaware of previously. Medawar had acknowledged his debt to Owen in his Nobel Lecture but, until I heard the details in Madison, I knew little about Owen or his work. I acknowledge Millard Susman, James Crow and Ray Owen for sharing images and information about this important time in reproductive immunology.

References