Discovery of Immunological Tolerance

4th Autumn School: Current Concepts in Immunology

Deutsche Gesellschaft für Immunologie
Bad Schandau

October 3, 2012

Tommy Douglas, Ph.D.
A Story of Chimeras, Cattle, Chickens, and Inbred Rodents
(but mostly cattle)
Four Pathways of Discovery Converged

#1 Immunogenetics
#2 Immunological Theory
#3 Transplantation Biology
#4 Tumor Immunology

Immunological Tolerance
Mosaic and Chimera

Archaeological Museum of Rhodes
Chimeras I Saw This Week

Frankfurt

Weimar

Erlangen
Twins: Monozygotic and Dizygotic

Eineiige Zwillinge

Abb. 20 Bildung von eineiigen und zwei e iigen Zwillingen

Zweieiige Zwillinge

F Moisl, Biologie I: Die Entstehung des Lebens, Deut. Taschenbuch Verlag, 1974
Humoral and Cellular Immunity

**Humoral Immunity**

- Specific
- Mediated by Antibodies
- Can be transferred from one individual to another by plasma or serum

**Cellular Immunity**

- Specific
- Not mediated by Antibodies
- Can be transferred from one individual to another, genetically compatible, individual only by living lymphocytes
Pathway #1: Immunogenetics
Where the Story Began:
The University of Wisconsin - Madison

www.wisc.edu/
Wisconsin: “America’s Dairyland”

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Surprising Finding #1

Endocrine Consequences of Twinning in Cattle
“The Theory of the Free-martin”

“In cattle a twin pregnancy is almost always a result of the fertilization of an ovum from each ovary; development begins separately in each horn of the uterus. The rapidly elongating ova meet and fuse in the small body of the uterus at some time between the 10 mm. and the 20 mm. stage.

The blood vessels from each side then anastomose in the connecting part of the chorion … if one is male and the other female, the reproductive system of the female is largely suppressed, and certain male organs even develop in the female. This is unquestionably to be interpreted as a case of hormone action.”

A Placenta with Vascular Anastomosis

L Lopriore, D Oepkes and FJ Walther, Early Human Devel. 87:595 (2011)
“The Theory of the Free-martin”

In one case in which the female co-twin of a male developed normally, Lillie noted that “the two [placentas] were entirely unfused; this case, therefore, constitutes an experimentum crucis.”

“In the case of the free-martin, nature has performed an experiment of surpassing interest.”

Surprising Finding #2

Hematologic and Immunologic Consequences of Twinning in Cattle
“Almost thirty years have passed since Lillie used the demonstrated union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the frequent reproductive abnormalities of the female twin. … Since Lillie’s paper…consequences other than endocrinological of nature’s experiment in parabiosis have, however, received little attention.”

One pair of **twins, who had different fathers**, had identical blood types, and each twin expressed blood group antigens that could not have been inherited from its own father or from its mother.

RD Owen, Science 102:400 (1945)
An Extraordinary Case: Twins with Different Fathers

Cartoon depicting the seminal observations of Dr. Ray Owen on dizygotic cattle twins. These twins (d and e), which were sired by two different bulls (A and B) mating with a Guernsey (C) shared a blood supply \textit{in utero}. When these cows grow up (D and E), they can accept grafts from each other. These observations led to Burnet’s hypothesis and the testing of experimentally induced unresponsiveness. Original drawing by \textbf{Willard Goodwin} provided by Dr. Ray Owen (farmer F in cartoon) with permission.
Other Evidence that Twin Cattle are Often Chimeras

• Among 80 pairs of bovine twins, most had identical blood types.

• Differential lysis, by antibody plus complement, revealed a mixture of two distinct types of erythrocytes in such twins.

• One bull, who was a twin, failed to transmit certain of his blood group antigens to any of his 20 offspring, suggesting that he was a genetic chimera.

RD Owen, Science 102:400 (1945)
“Immunogenetic Consequences of Vascular Anastomoses Between Bovine Twins”

Hematopoietic Stem Cells Must Have Been Exchanged During Gestation

“Since many of the twins in this study were adults when they were tested, and since the interchange of formed erythrocytes alone between embryos could be expected to result in only a transient modification of the variety of circulating cells, it is further indicated that the critical interchange is of embryonal cells ancestral to the erythrocytes of the adult animal. These cells are apparently capable of becoming established in the hemapoietic tissues of their co-twin hosts and continuing to provide a source of blood cells distinct from those of the host, presumably throughout his life.”

RD Owen, Science 102:400 (1945)
“Immunogenetic Consequences of Vascular Anastomoses Between Bovine Twins”

“If, therefore, the frequent identity of blood types in twin pairs can be explained neither as the result of monozygotic twinning [rare in cattle] nor as chance identity between fraternal twins, nor as a sum of these two factors, it is evident that some mechanism is operating to produce frequent phenotypic identity of blood types in genetically dissimilar twins. The vascular anastomosis between bovine twins, known to be a common occurrence, provides an explanation.”

RD Owen, Science 102:400 (1945)
“Immunogenetic Consequences of Vascular Anastomoses Between Bovine Twins”

These results suggested a diagnostic test:

“A heifer [young cow] whose blood type is the same as her twin brother’s will very probably be a freemartin, while a difference in even a single antigen between twins of opposite sex may indicate that vascular anastomosis did not occur, and therefore that the heifer will be normal.”

This prediction was later confirmed by William Stone et al.

RD Owen, Science 102:400 (1945)
W Stone, C Stormont and MR Irwin, J. Animal Science 11:744 (1952)
“Immunogenetic Consequences of Vascular Anastomoses Between Bovine Twins”

What will the future hold?

“Several interesting problems in the fields of genetics, immunology, and development are suggested by these observations. Most of them are still largely speculative and will not be considered here.”

RD Owen, Science 102:400 (1945)
Erythrocyte Mosaics Among Bovine Twins and Quadruplets

A similar situation occurs in multiple births of higher order

“No twin has shown more than two cell types, but a mixture of three cell types has been demonstrated in each member of a set of quadruplets.”

What other tissues might contain cells of hematopoietic origin?

“The extent to which tissues other than blood may depend upon primordial cells circulating in embryos, and may therefore show a similar mosaicism associated with multiple births in cattle, remains an open question.”

RD Owen, Genetics 31:227 (1946)
A similar situation occurs in multiple births of higher order (cont.)

“Descriptive data on a set of living bovine quintuplets, one modified heifer and four bulls, suggest that the animals developed from five different fertilized eggs. Their bloods, however, gave identical results when tested for inherited cellular antigens.”

“The homogeneity of these quintuplets with respect to their relative proportions of A and “non-A “ cells indicates a very thorough intermixture of at least two different cell types among the quintuplets as embryos.”

“This result is consistent with the conclusions : (1) that placental anastomoses had given all five embryos a common circulation; (2) that wholesale exchange and intermixture of circulating cells among the embryos was therefore effected; and (3) that the exchanged cells became established in the hemapoietic tissues of the five animals, so that the circulating erythrocytes, the cellular descendants of these exchanged elements, now represent a genetic mosaic, similar in all of the quintuplets.”

RD Owen, HP Davis and RF Morgan, J. Heredity 37:291 (1946)
Earlier Studies of Blood Groups in the Rat

“In 1946, primarily to implement an investigation of whether the natural parabiosis of fetal twin cattle could be mimicked by surgical parabiosis of young rats, some reagents were developed to recognize individual differences in rat red cell antigenic specificities….Reagents for the C-D alternative…proved useful for early studies of RBC chimerism following parabiosis (1956), embryonic injection (1956), and bone marrow injection after X irradiation (1955,1957).”

Bringing the Study of Tolerance “Into the Lab”

Enormous Experimental Advantages are offered by the use of Inbred Strains:

![Graph showing probability of homozygosity over generations.](image)

Figure 3.4. Probability of homozygosity at locus $A$ during the first 20 generations of brother-sister mating (inbreeding).

Antigenic Characteristics of Rat Erythrocytes, and Their Use as Markers for Parabiotic Exchange

“A very highly inbred strain of Wistar albino rat is heterogeneous for cellular characteristics. Each of the seven other inbred strains tested is homogeneous. – These erythrocyte differences provide convenient markers for an investigation of cellular exchange in parabiosis. Data have been collected covering such subjects as: time of first detectable exchange; extent of exchange; the approach to equilibrium of the two cell-types; the appearance of incompatibility and the course of auto-disjunction; the distribution of injected foreign cells; and time of persistence of exchanged cells after surgical disjunction., a measure of the life-span of rat erythrocytes.”

RD Owen, Genetics 33:623 (1948)
Bringing the Study of Tolerance “Into the Lab”

Erythrocyte Antigens and Tolerance Phenomena

“Our failure to mimic the twin cattle phenomenon with parabiotic rats was interpreted as dependent on the age of the individuals united; we worked only with rats somewhat more than two weeks after birth. ... In 1952-3, S. H. Ripley, then a graduate student conducting his minor problem research in my laboratory, was able to demonstrate that injections of foetal liver and spleen cells into the chorionic vessels of foetal rats resulted in the establishment of a persistent erythrocyte mosaicism.”

The First Case of Human Blood Cell Chimerism is Discovered

A Human Blood-Group Chimera

“Mrs. McK., a donor aged 25, gave her first pint of blood in March of this year. When the blood came to be grouped it seemed to be a mixture of A and 0 Cells… When asked if she were a twin, Mrs. McK., somewhat surprised, answered that her twin brother had died 25 years ago at the age of 3 months… It is strange that we should be able to group completely this twin 25 years after his death.

The First Case of Human Blood Cell Chimerism is Discovered

Mrs. McK, a British blood donor, was found to have an unusual mixture of blood cells. Investigation revealed that she was born a twin, but her brother had died in infancy. Cells from the brother, that had been transplanted into Mrs. McK's body when the twins were embryos, still survived.

"K" blood group = Kell

RD Owen, Engineering and Science (Caltech) June 1959:16
The First Case of Human Blood Cell Chimerism is Discovered


"K" blood group = Kell
The First Case of Human Blood Cell Chimerism is Discovered

• Mrs. McK, who was genetically type O, demonstrated **immunological tolerance** because she produced antibodies against type B red blood cells (as expected), but **not** against type A red blood cells.

• Although Mrs. McK was genetically a secretor (into saliva) for ABO antigens, she only secreted only O substance, and not A substance, due to her status as a **chimera**. (The A gene from her twin brother was only expressed in her blood, not in her salivary glands.)

• Mrs. McK gave birth to a child – she was **not** a “**freemartin**.”

Pathway #2:

Immunological Theory
Frank Macfarlane Burnet

F.M. Burnet in the laboratory in the early 1950's. He was experimenting on influenza virus genetics, using the developing hen's egg. Copyright © Walter and Eliza Hall Institute

www.nobelprize.org/nobel_prizes/medicine/articles/wehi/index.html
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THE
PRODUCTION OF ANTIBODIES

By
F. M. BURNET, M.D., F.R.S.
and
FRANK FENNER, M.D.

Second edition

MELBOURNE
MACMILLAN AND COMPANY LIMITED
HEAD OFFICE: LONDON
1953

Second Edition Published 1949
Development of “Marker” Recognition in Embryonic Life

“If in embryonic life expendable cells from a genetically distinct race are implanted and established, no antibody response should develop against the foreign cell antigen when the animal takes on independent existence. What is at present a unique natural example of such a circumstance has been described. In the course of detailed studies of blood groups in cattle and their inheritance, Owen and his colleagues...studied examples of multiple births in which there had been a common placental circulation although the embryos were of multiovular origin. In these instances the normal segregation of blood group character did not occur. Two calves from the same birth would each show two coexisting serological types of cell when by ordinary genetic rules each should have one only. Under normal circumstances the blood cells of one calf should have been antigenic if injected into the circulation of the other; when the cross transfusion takes place in embryonic life no immunological response follows either then or subsequently. A very interesting field for direct experimentation is opened up by this finding; particularly if the same type of phenomenon can be induced by intravenous inoculation of foreign embryonic blood cells in chick embryos.”

FM Burnet and F Fenner, The Production of Antibodies, 2nd Ed., Cambridge, 1949
Pathway #3:
Transplantation Biology

Pathway #4:
Tumor Immunology
Are Littermates Identical?: A Practical Problem

Four calves born in Aylsham, Norfolk in 1949 appeared to be identical quadruplets

HP Donald et al., Heredity 5:135 (1951)
“These calves [born in January 1949] are remarkable, not only because quadruplets are unusual in cattle, but also because they were born alive, and were all of the same sex. Furthermore, they appear to be the first recorded set judged to be of monozygotic origin. … It would have been interesting to study the blood groups, but this was not possible.”
Peter Medawar

Are Two Littermates Identical?: A Practical Problem

Medawar – The Uniqueness of the Individual

My colleagues and I have shown that Burnet and Fenner’s prediction is true, without qualification, of the antigens which are responsible for transplantation immunity. We too began our work on cattle. In 1948, while attending an International Congress of Genetics at Stockholm, I was invited by Dr. H.P. Donald to help solve the important problem of distinguishing with complete certainty between identical and non-identical twins in cattle. In principle, nothing could be easier. Skin grafts were to be exchanged between the twins a few weeks after their birth. If the homografts survived, the twins could be classified as identical; if not, as non-identical, i.e. dizygotic.

PB Medawar, The Uniqueness of the Individual, Basic Books, 1957
Surprising Finding #3

Consequences of Twinning in Cattle for Skin Graft Rejection
The Use of Skin Grafting to Distinguish Between Monozygotic and Dizygotic Twins in Cattle

“An investigation has been made of the value of skin grafting for discriminating between monozygotic and dizygotic cattle twins, in accordance with the general rule that skin grafts exchanged between genetically dissimilar mammals are rapidly destroyed.

...skin homografts were exchanged between the members of 7 different pairs of allegedly dizygotic female twins. In not a single animal did any of the homografts undergo the rapid, clear-cut breakdown that might have been anticipated from the results obtained when grafts were exchanged between unrelated animals.

...dizygotic twins have identical red cell antigens and uniformity of red cell antigens between sibs other than twins is rare. Owen has produced evidence that the blood of dizygotic twin cattle may contain a mixture of red cells of different antigenic type... The dizygotic twin calf at birth is already, in fact, a genetical chimera. The experiments described in this paper provide direct confirmation of Owen's hypothesis.

We are proposing to study the possible desensitisation of mouse embryos by inoculated foreign cells...”

D Anderson, RE Billingham, GH Lampkin and PB Medawar, Heredity 5:379 (1951)
By the 1940s, Several Strains of Mice Were Already Highly Inbred

Tolerance to Homografts, Twin Diagnosis, and the Freemartin Condition in Cattle

“All cattle we tested were in some degree tolerant to homografts from their two-egg twins, even when the twins were of unlike sex; the majority were, within the terms of our experiments, completely tolerant.

The mutual exchange of skin grafts therefore failed, in the majority of trials, to distinguish between one-egg and two-egg twins.

The proportion of cattle of two-egg twin birth that show complete tolerance to grafts from their respective twins corresponds closely with the proportion of females in two-egg twin pairs of unlike sex that reveal some degree of infertility or sexual abnormality. It is therefore argued that homograft tolerance and the freemartin state share at least one necessary causal condition in common. This condition is presumed to be the confluence of the foetal circulations of cattle twins.”

[Three years later, M Simonsen reported that a kidney transplanted from one cattle twin to the other continued to function for five months.]

RE Billingham, GH Lampkin, PB Medawar and H LL Williams, Heredity 6:201 (1952)
M Simonsen, Nature 175:763 (1955)
‘Actively Acquired Tolerance’ of Foreign Cells

“It is one of the predictions of Burnet and Fenner’s theory of immunity that the exposure of animals to antigens before the development of the faculty of immunological response should lead to tolerance rather than to heightened resistance.

The experiments to be described in this article provide a solution – at present only a ‘laboratory’ solution – of the problem of how to make tissue homografts immunologically acceptable to hosts which would normally react against them.”

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
‘Actively Acquired Tolerance’ of Foreign Cells

Preliminary Experiments with Chickens

“The embryonic chick is particularly well suited to experiments which make use of cellular inoculation, because the intravenous route is so easily accessible.”

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
‘Actively Acquired Tolerance’ of Foreign Cells

Preliminary Experiments with Chickens (cont.)

...we have obtained successful results by ...transfusing...whole blood from an 11-12 day old embryonic Rhode Island Red donor into a chorioallantoic vein of a White Leghorn embryo of the same age. Fourteen days after hatching, a test-graft of skin was transplanted to the recipient from its original donor*.

In seven such trials, five grafts showed prolongation of survival; of these, three succumbed within fifty days to the accompaniment of very much subdued inflammatory changes, and two still survive, with normal growth of red feathers, to the present time (125 days).”

[*Due to the lack of inbred strains, it was necessary that the very same donor of tissues or blood also had to be used as the donor of the test graft.]

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
A Rhode Island Red Graft 282 Days After Transplantation to Its 6-Day-Old White Leghorn Parabiotic Partner*

RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
* “Artificial…Twinning (‘Parabiosis’) in 10- to 11-Day-Old Chicken Embryos. The Shell and Shell Membranes Have Been Removed Over a Circular Area in Both Eggs to Expose the Chorio-Allantoic Membranes.”

RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
‘Actively Acquired Tolerance’ of Foreign Cells

Experiments with Mice

“…a **CBA** female in the 15 -16 day of pregnancy by a CBA male was anaesthetized …and its body wall exposed by a median ventral incision of the skin. …six foetuses were brought into view through the body wall. Each was injected intra-embryonically … with a suspension of [**Strain A**] adult tissue cells.” [finely chopped testis, kidney, and spleen].

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
‘Actively Acquired Tolerance’ of Foreign Cells

Experiments with Mice (cont.)

“Eight weeks after their birth…each member of the litter was ‘challenged’ with a skin graft from an adult A-line donor. The grafts on … three … resembled autografts in every respect except their donor-specific albinism.”

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
The tolerated grafts were still antigenic: intraperitoneal inoculation of the recipients with lymph node fragments from CBA mice that had been actively immunized against strain A skin caused rapid rejection of the previously tolerated grafts. [NA Mitchison had very recently reported the adoptive transfer of transplantation immunity against tumor cells by immune lymph nodes.]

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
Experiments with Mice (cont.)

If grafts are removed from hosts that have tolerated them and transplanted to normal mice of the host’s strain, they are rejected.

Some treated animals showed only partial tolerance, as judged by how long the test-grafts were retained.

Tolerance is immunologically specific: mice rendered tolerant to strain A grafts were not tolerant to grafts from a different inbred strain [AU].

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
RE Billingham, L Brent and PB Medawar, Phil. Trans. Roy. Soc. 239:357 (1956)
‘Actively Acquired Tolerance’ of Foreign Cells

Experiments with Mice (cont.)

Many different types of tissue cells are capable of conferring tolerance to homografts of skin.

It is much more difficult to induce tolerance in newborn mice than in embryonic mice.

Fertility of the treated mice was unimpaired. [So, the induction of tolerance was not causally related to the freemartin condition.]

Offspring of the tolerant animals are not themselves tolerant.“Actively acquired tolerance may not be a wholly artificial phenomenon. We are inquiring into the possibility that it may occur naturally by the accidental incorporation of maternal cells into a foetus during normal development.”

RE Billingham, L Brent and PB Medawar, Nature 172:603 (1953)
Nobel Prize

In 1960, the importance of this work was recognized by the Nobel Prize in Physiology or Medicine, which was awarded "for discovery of acquired immunological tolerance".
90th Birthday Celebration for Ray Owen

June Owen and Ray Owen

October 2005
90th Birthday Celebration for Ray Owen

Leonard Herzenberg, Leslie Brent, and Carol Brent

October 2005
90th Birthday Celebration for Ray Owen

A History of Transplantation Immunology

Leslie Brent BSc PhD FInst Biol Hon MRCP
Emeritus Professor of Immunology
St Mary's Hospital Transplant Unit
Paddington, London, UK

To Fiona

With very best wishes.

Leslie

29.10.2005

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October 2005
90th Birthday Celebration for Ray Owen

RAY DAVID OWEN (1915–)

Tommy, all the best!
Ray

Tommy Douglas

October 2005
Further Reading

For a Current Review About the History of Research on Immunological Tolerance:

For an Interview with Ray Owen, Describing His Career in Science and Education:
California Institute of Technology Archives - Oral History Project
http://resolver.caltech.edu/CaltechOH:OH_Owen_R