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## The Discovery of Immunological Tolerance: Now More Than Just a Laboratory Solution

Kathryn J. Wood, Andrew R. Bushell, and Nick D. Jones

The discovery of acquired immunological tolerance demonstrated unequivocally that immune responses against a defined set of Ags could be abolished, or at least attenuated, using a biological approach. This important series of experiments by Billingham, Brent, and Medawar (1), using a transplantation model, not only set the stage for future advances in clinical transplantation, but also, at a more fundamental level, defined the principles underpinning the immunological basis of tolerance to self-Ags.

The study reported in *Nature* in 1953 was stimulated by the work of Ray Owen (2), who was the first to observe the phenomenon of immunological tolerance in vivo when he documented the coexistence of two types of erythrocytes in the blood of dizygotic cattle twins. Each twin not only had its own red blood cells but also had a second set of cells that were derived from the other twin through a shared placenta. Owen concluded that the twins must have exchanged the erythrocytes or their precursors in utero and that the cells survived as the calves grew into adults. This naturally acquired lack of immune reactivity to the foreign red blood cells and the Ags that they expressed was a clear example of immunological tolerance in action; this led Burnet and Fenner (3) to predict that exposure to an Ag early in life would induce specific tolerance to that Ag, as evidenced by the absence of an immune response to the same Ag later in life.

Independently, and for different reasons, Billingham and Medawar were examining the fate of skin allografts in young cattle, with the objective of devising a test for distinguishing between fraternal and identical twins. To their surprise, they found that skin grafts transplanted from one twin to the other were accepted, irrespective of the origin of the twins (4). To take this to the next level, Medawar argued that if Burnet and Fenner's hypothesis to explain Owen's observations was correct, it should be possible to introduce Ags to the developing immune system and induce immunological tolerance. The experiments in mice and chickens (1) reviewed below, together with those of Hasek (5), who at the same time was studying parabiosis in chickens, demonstrated that this was the case.

The aims of Billingham, Brent, and Medawar were to develop an experimental system to demonstrate or "prove" that tolerance to a known Ag could be induced deliberately in vivo and, once this was established, to study the mechanisms responsible.

In the paper, they state that they wanted to make tissue allografts, or homografts as they were then known, "acceptable to hosts which would normally react against them." The "principle" or hypothesis that they wished to test was that "mammals and birds never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells to which they have been exposed sufficiently early in foetal life." They argued that if a fetal mouse from one inbred strain was inoculated in utero with a suspension of living cells from an adult mouse from a different inbred strain, as the fetal mouse grew into an adult, it would be completely or partially tolerant to a skin graft from the original cell donor. In other words, this experimental system would mimic the natural development of tolerance they had observed in the cattle twins. Interestingly, they regarded the acquisition of tolerance through this approach as the inverse of actively acquired immunity, such as occurs following immunization, the key being the introduction of foreign Ags into a developing immune system, as opposed to a fully formed, adult immune system.

The experimental design used CBA mice as recipients and a second genetically distinct inbred strain A as cell and skin graft donors. Adult unmanipulated CBA mice rejected A strain skin grafts within 11 d, as expected; if a second A strain graft was transplanted at any time up to 60 d later, it was rejected by the mice now sensitized to strain A Ags much more rapidly, within 6 d. This accelerated immune response could be transferred to a normal CBA adult by implanting pieces of lymph node into the peritoneal cavity, thus demonstrating that lymphocytes, rather than serum factors, were responsible for this "active immunisation" against the foreign Ags of the A strain. Next, they injected each of the six fetuses in a CBA female mouse in day 15 or 16 of pregnancy with 10  $\mu$ l of A strain adult tissue (experiment 37 reported in detail in the article). Five offspring were born; 8 wk after birth, they challenged each littermate with an A strain skin graft. The skin graft survived in three of the five mice, with two accepting the transplant long-term; it was rejected in the other two mice. Skin grafts from a different mouse strain, AU, were always rejected by the mouse exposed to A strain cells in utero.

Importantly, Billingham, Brent, and Medawar found that the induction of tolerance using this strategy was immunologically specific to the foreign histocompatibility Ags introduced into the developing immune system, that timing was critical because delaying the injection of the cells until after birth was less effective or ineffective, and that it was not tissue specific. Another critical point that is sometimes overlooked is that the state of tolerance induced was not an all-or-nothing phenomenon, with "every degree" of tolerance represented in their experiments.

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Investigating the mechanism responsible for this acquired state of immunological tolerance, they showed that the tolerated skin grafts had not lost their ability to be rejected, because the implantation of chopped fragments of lymph nodes from normal CBA mice that had been actively immunized against the A strain resulted in the rapid rejection of the tolerated grafts. Thus, the tolerance induced could be overcome by Ag-experienced donor alloantigen-reactive leukocytes. Interestingly, they also observed that when a tolerant mouse bearing an A strain graft was challenged with an AU graft, rejection of the AU graft could induce a severe crisis in the first A strain transplant. They knew that strains A and AU shared some Ags but could not understand this observation, because they believed that the tolerant mouse should have been tolerant of the shared Ags. They suggested that this might be linked to alloantibody production, an interesting observation in light of the clinical protocols for tolerance induction reported recently (6).

The impact of this work, together with that of Owen and Hasek (2, 5), which defined the principles underpinning the current understanding of the immunological basis of tolerance to self-Ags and foreign Ags cannot be overstated and demonstrates the power of transplantation models for exploring basic as well as applied questions in immunology. Clonal deletion of T cells reactive to self-Ags in the thymus during the development of the immune system, together with the need for a peripheral mechanism to control any escaping self-reactive clones, are now established as the mechanisms that prevent the development of autoimmune diseases (7, 8) and allow the induction of transplantation tolerance (9, 10).

In the opening sentence of the *Pillars of Immunology* article (1), Billingham, Brent, and Medawar acknowledged that their findings were only a “laboratory solution” to the issue of tolerance in clinical transplantation. Clearly, these ideas were

the impetus for a concerted effort over the last 55 y to develop strategies for inducing tolerance in clinical transplantation, such that today some patients who have undergone a transplant have a functioning transplant without taking lifelong chemical immunosuppression (11). Moreover, strategies to induce tolerance in clinical transplantation that are based upon the principles of the Medawar protocol have been developed to allow transplantation tolerance to develop in selected groups of transplant recipients (6, 12).

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