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MILESTONES IN TRANSPLANTATION: THE STORY SO FAR

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NOTE: The milestones in the following material were discussed at a historical concensus conference held at the University of California, Los Angeles (UCLA) to which 11 early workers in transplantation were invited: Leslie B. Brent (London), Roy Y. Calne (Cambridge, Eng), Jean Dausset (Paris), Robert A. Good (St. Petersburg, Fla), Joseph E. Murray (Boston), Norman E. Shumway (Palo Alto), Robert S. Schwartz (Boston), Thomas E. Starzl (Pittsburgh), Paul I. Terasaki (Los Angeles), E. Donnall Thomas (Seattle), Jon J. van Rood (Leiden). Each man provided personal reflections which have been published in a special issue of the World Journal of Surgery (Volume 24:755-843, 2000). However, the ultimate objective was to reach consensus on the key historical discoveries prior to 1975 that eventually allowed clinical transplantation to become a feasible and practical form of therapy. Carl Groth of Stockholm was invited to be the Chairman for these consensus deliberations, and to prepare the executive summary (Groth et al, 2000). Concensus landmarks in the summary were restricted to those made at least a quarter of a century ago. Although advances in the 1975-2000 period were not formally reviewed, the ones alluded to here appear destined for milestone status.

The concept of transplanting animal or human tissues and organs to patients is almost as old as recorded history (Bhandari and Tewari, 1997). However, the first enduring contribution was the technology of blood vessel anastomosis developed by Carrel (1902) (see Table 1). Carrel recognized that transplanted organ allografts and xenografts were not permanently accepted, although he did not know why.

THE TECHNICAL CHALLENGE

The Kidney

Attempts at clinical renal xenotransplantation by vascular anastomoses were undertaken at the beginning of the twentieth century in France and Germany using pig, sheep, goat, and subhuman primate donors. None of the kidneys functioned for long, if at all, and the unmodified human recipients died a few hours to 9 days later. No further clinical xenotransplantations were tried again until chimpanzee (Reemstsma et al, 1964) and baboon kidney xenografts were transplanted to human recipients under immunosuppression (Starzl et al, 1964d).

The first known attempt at transplantation of an organ allograft was reported from Kherson (the Ukraine) in 1936 by Yu Yu Voronoy (1937); an English translation of the article has been provided by Hamilton and Reid (1984). The kidney, which was removed from a cadaver donor never functioned. This

was not surprising in view of multiple adverse factors: ABO incompatibility between the donor and recipient, a 6 hours delay at room temperature between donor death and kidney removal, and the recent suicide attempt of the recipient by mercury ingestion. In 1951, systematic clinical trials of kidney allotransplantation in unmodified human recipients were undertaken in France by Kuss, Teinturier and Milliez (1951), Dubost et al (1951), and Servelle, Soulie, and Rougeulle (1951). Most of the kidneys were obtained from criminals immediately after their execution by the guillotine, and some briefly excreted urine.

The first live donor kidney transplantation was performed in Paris by Michon et al (1953), using the extraperitoneal pelvic procedure developed by Kuss. This mother-to-son transplantation resulted in prompt kidney function that continued for three weeks before the allograft was rejected by the unmodified recipient. Kuss's procedure has been used worldwide ever since with an outstanding record of safety and reliability.

In the meanwhile, 9 kidney allotransplantations were performed between 30 March 1951 and December 3, 1952 in patients whose pre- and post-transplantation dialysis was at the Peter Bent Brigham Hospital ("The Brigham") in Boston (Hume et al, 1955). In the first of these operations,

performed by L. H. Doolittle in Springfield, Massachusetts, the allograft was transplanted to the vacated renal fosa of the recipient after removal of the native organ. The next 8 transplantations were performed at the Brigham. All 8 of these allografts were placed by David Hume in the recipient anterior thigh. Some of the recipients received adrenal cortical steroids, and one of the transplanted kidneys produced urine for 5 months.

In December 1954, kidney transplantation from an identical twin donor recipient was carried out by Joseph E. Murray at the Brigham Hospital in Boston (Murray, Merrill, and Harrison, 1955; Merrill et al, 1956). It was known from earlier research by plastic surgeons that skin grafts from identical twins were not rejected. To test genetic identity, reciprocal skin grafting was carried out prior to the kidney transplantation. Despite 82 minutes of warm ischemia, the isograft functioned immediately and for the next 25 years, until the death of the recipient from atherosclerotic coronary artery disease. Although the identical twin kidney transplantations did not provide fundamental new information about transplantation immunology, the cases exemplified the potential power of transplantation.

The Extrarenal Organs

Using vascular surgical techniques, animal research in transplantation was most highly focused on the kidney for most of the first half of the 20th century. The extrarenal vacuum rapidly was filled between 1958-1960 with the development in several laboratories of canine models with which to study all of the intra-abdominal and thoracic organs. Although each organ presented specific technical and physiologic issues, the core problems of immunosuppression, tissue matching, and allograft preservation eventually were worked out mainly with the kidney or/and the liver and applied to other organs with minor modifications.

THE SEMINAL TURNING POINTS

The modern history of transplantation could be written from the vantage point of the first successful use in humans of allografts of the various organs and of bone marrow (Table 2). However, a more accurate and complete picture can be obtained by reviewing how it was learned to harness destructive immunity enough to allow allograft survival. After Medawar's demonstration that rejection is an immune reaction (1944), the feasibility of transplanting allografts hinged on 2 observations. The first was the discovery in 1953 by Billingham, Brent and Medawar (1953, 1956) that chimerism-associated neonatal tolerance could be induced in intrauterine and neonatal mice by the infusion of donor hematolymphopoietic

cells (i.e. splenocytes and bone marrow). The second seminal turning point was the recognition that human organ allografts were inherently tolerogenic when transplanted to immunosuppressed recipients (Starzl, Marchioro, and Waddell, 1963b).

Unfortunately, these 2 sets of observations led to a Y in the road beyond which successful engraftment of bone marrow was explained by donor leukocyte chimerism-dependent mechanisms, whereas organ engraftment was attributed to chimerism-independent mechanisms. This egregious error precluded genuine insight into the immunology of transplantation for nearly 3 decades and resulted in a systematic misinterpretation of research studies in transplantation (Starzl, 2000). That a mistake of such magnitude could have been perpetuated for so long without a single challenge in the scientific literature is truly remarkable. This can be explained in part by the primitive state of immunology (see next section) at the time the false dogma became imbedded in the scientific literature and textbooks.

THE ASCENDENCY OF IMMUNOLOGY

The foundation of immunology had been laid at the turn of the century by the piecemeal discovery of the different components of the immune response and of the role of immunity

in the defense against infectious disease. This wave of advances resulted in a large proportion of the first 30 Nobel prizes (Table 1). The big leaps were succeeded by a period of "consolidation" that was reflected by a gap of 30 years between the 1930 Nobel prize for Landsteiner's discovery of ABO groups and the 1960 prize which was awarded to Burnet and Medawar (see Table 1). Burnet with Fenner (1949) had initially postulated that an individual produced antibodies only to those antigens to which (s)he had been exposed. Over the next decade, this clonal selection hypothesis was validated and extended by Burnet (1959) to cellular immunity, thus providing a conceptual framework for the ontogeny of the immune system as well as for its function.

An important supporting strut in Burnet's hypotheses was the earlier study by Owen (1945) of freemartin cattle, the bovine equivalents of human fraternal twins. Owen observed that permanent hematopoietic chimerism developed reciprocally in calves whose placentas had fused, thereby allowing intrauterine circulatory exchange. Working with Medawar, Anderson et al (1951) subsequently showed that these cattle also had reciprocal tolerance to skin grafts. The authors speculated that actively acquired tolerance could be induced deliberately by mimicking the conditions of cross circulation. When the neonatal tolerance experiments of Billingham, Brent, and Medawar (1953,1956) upheld the prediction, the

observations were viewed as formal proof of the clonal selection theory of Burnet.

THE BILLINGHAM-BRENT-MEDAWAR EXPERIMENTS

In the original Billingham-Brent-Medawar experiments (1953), acquired tolerance to skin allografts was induced in fetal and neonatal mouse recipients whose immunologically immature immune system was unable to reject the infused splenocytes from adult donors. In later experiments, bone marrow cells were used (1956). The inoculated animals that survived to adult life had circulating donor leukocytes and developed specific non-responsiveness to donor strain skin allografts, while evolving normal reactivity to third party grafts. By 1957, however, it had been learned that the engraftment of immunocompetent donor leukocytes in immunologically defenseless recipients caused graft-versus-host disease (GVHD) that was avoidable or controllable only when there was a close genetic relationship between donor and recipient (i.e. a good "tissue match") (Billingham and Brent, 1957 and 1959; Simonsen, 1957).

In a logical extension of these experiments, adult mice were preconditioned for bone marrow transplantation with supralethal total body irradiation [TBI]). With engraftment of the donor bone marrow cells in these animals, the result was the same in principle as that achieved a decade later with

human bone marrow transplantation to cytoablated recipient. However, the requirement for a good tissue match in mice (Trentin, 1956) applied equally to humans (Mathe et al, 1963; Gatti et al, 1968; Bach et al, 1968). Mathe was the first to achieve prolonged survival after engraftment of allogeneic human bone marrow cells, but in spite of good histocompatibility between multiple familial donors and the cytoablated recipient, chronic graft-versus-host disease (GVHD) developed in this patient and caused his death after 2 years. Finally, in 1968, bone marrow cells were successfully transplanted from familial donors into 2 recipients whose immune deficiency diseases made cytoablation unnecessary (Gatti et al, 1968; Bach et al, 1968). Both patients are alive and well 32 years later.

IMMUNOSUPPRESSION FOR ORGAN TRANSPLANTATION

Once rejection was identified by Medawar (1944) as an immunological event, weakening the recipient response with TBI or with immunosuppressive drugs, became a logical strategy for mitigating or preventing the immune reaction. Cortisone, which did not depress bone marrow, was the first drug to prolong the survival of rabbit skin allografts (Billingham, Krohn, and Medawar, 1951), but the effect was modest. In contrast, observations in chickens reported by Cannon and Longmire (1952) were of exceptional significance. However,

the importance of this work was not recognized until many years later.

In control experiments, Cannon and Longmire showed that freshly hatched chicks permanently accepted skin allografts from different adult breeds in 6% of experiments. When a course of cortisone treatment was given, this incidence rose to over 20% without an increased mortality. The critical observation was that the skin allograft survival was of lifetime duration: i.e. continued after discontinuance of the steroid course. This finding presaged the discovery in Denver a decade later that organs were inherently tolerogenic in patients treated with azathioprine and dose-maneuverable prednisone (Starzl, Marchioro, and Waddell, 1963b). Because the Cannon/Longmire studies had been long since passed over, however, they did not alter the pessimistic attitudes prevalent at the time about the feasibility of clinical organ transplantation.

Total Body Irradiation (TBI)

Instead, it was widely believed by the late 1950s that successful organ transplantation would not be possible without establishing donor leukocyte chimerism by the concomitant or preceding engraftment of donor bone marrow cells as had been shown to be feasible in cytoablated mice (Main and Prehn, 1955; Trentin, 1956). In practice, this approach proved to be

impossible in large outbred animals (Hume et al, 1960). With the dog model, only a single irradiated beagle recipient survived for as long as 70 days following combined bone marrow and kidney transplantation (Mannick et al, 1959).

Despite this discouraging record in animals, Murray et al attempted the combined procedure at the Peter Bent Brigham Hospital (Boston) in 2 TBI-conditioned patients, both of whom died in less than a month. The next 10 human recipients in this trial were conditioned in 1958-60 with sublethal TBI, followed by kidney transplantation alone (Murray et al, 1960 and 1962). All but one of the patients also died within one month. In the exceptional case, however, the irradiated recipient of a fraternal twin kidney survived for more than 20 years before dying of a malignant tumor. It was the first example in the world in any species including humans of successful organ transplantation from a genetically non-identical donor.

It was suspected initially that placental fusion and cross circulation between the twins may have occurred in utero (as with Owen's freemartin cattle). The same reservation pertained to a second extended survival following fraternal twin kidney transplantation in Paris 5 months later (Hamburger et al, 1959). In the succeeding 3 years, however, the issue was settled by 4 more examples of survival $\geq$ one year in

Paris. Two of these irradiated patients received kidneys from non-twin family members (Hamburger et al, 1962). The kidneys in the other 2 cases were from non-related donors (Kuss et al, 1962) (Table 3). Because none of the 6 kidney allograft recipients who had $\geq$ one year survival had been given adjunct donor bone marrow cells, it was concluded that donor leukocyte chimerism was not a necessary condition for successful kidney transplantation.

Pharmacologic Immunosuppression

Because the failure rate using TBI was overwhelming, the prospects for developing kidney transplantation as a clinical service remained grim until the end of 1962. A sea change began with the testing of drugs whose myelotoxicity initially prompted their use as a substitute for TBI. In 1963, Willard Goodwin of Los Angeles belatedly reported a case of a mother-to-daughter kidney transplantation that had been carried out in September 1960, after first producing severe bone marrow depression with large doses of methotrexate and cyclophosphamide. During the 143 days of survival, the recipient developed several rejections that were reversed with prednisone (Goodwin et al, 1963).

Although this was the first example of extended survival of a human kidney recipient without the use of TBI, the case did not significantly impact the field because its existence

was not generally known. In the meanwhile, it had been learned in studies of rabbit skin transplantation (Schwartz and Dameshek, 1960; Meeker et al, 1959) and of kidney transplantation in dogs (Calne 1960, 1961a; Zukoski et al, 1960) that the drug 6-mercaptopurine (6MP) and its imidizole derivative azathioprine, were immunosuppressive at submyelotoxic doses. Both agents permitted only about 5% long term survival of canine kidney allografts (Murray et al, 1962), but the transplanted kidneys in some of the long-surviving animals continued to function long after discontinuance of immunosuppression (Pierce and Varco, 1962; Zukoski and Calloway, 1963; Starzl, 1964a; Murray et al, 1964). The observation was reminiscent of the finding in newly hatched chicks treated with a short limited course of cortisone that had been described a decade earlier (Cannon and Longmire, 1952).

Realizing that neither 6-MP nor azathioprine alone would permit more than an occasional clinical success, Calne and Murray (1961b) tested azathioprine in combination with other myelotoxic drugs at the Brigham canine laboratory. Prompted by the personal communication from Goodwin about the effect of steroid therapy (Murray, 1999 [see earlier]), prednisone also was added to azathioprine. When the azathioprine-prednisone combination appeared to be no more effective than azathioprine alone (Calne, 1961a, Calne et al, 1962), the decision was made

to use azathioprine with the myelotoxic agents azaserine and actinomycin for the Boston clinical trials. Only one of the first 10 drug-treated kidney recipients survived. The exceptional patient received a non-related kidney on April 5, 1962, and remained dialysis-free for 17 months until the allograft was lost to chronic rejection. Thus, this patient became the seventh one-year kidney allotransplant survivor in the world. More importantly, he was the first to achieve this milestone without TBI (Table 3).

With the late rate of success, however, kidney transplantation was still viewed with pessimism. This was relieved by 2 observations at the University of Colorado, first in canine kidney recipients and then in a series of 10 consecutive human cases compiled in 1962-63. The addition of high doses of prednisone to baseline therapy with azathioprine resulted not only in reversal of established rejection, but also in the variable induction of donor specific tolerance (Starzl, Marchioro, and Waddell, 1963b). Nine of the 10 human recipients had prolonged survival and 2 remain alive today with excellent function of their original grafts in their 38th post transplant year. These 2 patients (cases 2 and 3 in the original series), who bear the longest continuously functioning allografts in the world, have been free of all immunosuppression for 5 and 36 years respectively.

These results, and especially the easily taught treatment principles with which they were accomplished, fostered a whirlwind of activity in the United States and Europe. While dozens of new kidney transplant centers were established, the mechanisms by which the allografts had been "accepted" remained unknown. However, the conclusions that the kidney allografts had induced variable donor specific tolerance, and that engraftment depended on alterations in the transplanted organ plus a loss of specific responsiveness by the recipient (Starzl, Marchioro, and Waddell, 1963b; Starzl, 1964a) has stood the tests of time and of experimental verification.

These conclusions continued to generate controversy for 3 decades. Commenting on mechanisms, Medawar (1965) suggested that a progressive replacement of the vascular endothelium of the graft vessels by endothelium of host origin may have been made possible by weakening the allograft reaction with immunosuppression. Neither this hypothesis nor the suggestion that "a protective" host antibody had come to cover the endothelial layer (attributed to Calne by Medawar, 1965) has proved to be correct.

The enigmatic phenomena of the reversal of rejection and the development of variable donor specific tolerance observed after kidney transplantation were soon demonstrated with other organs. Furthermore, canine recipients of orthotopic liver

allografts self-induced tolerance under short term azathioprine therapy much more frequently than renal allografts (Starzl et al, 1965a). Soon thereafter, examples of spontaneous engraftment and self-resolving rejection crises in the absence of treatment were reported following liver transplantation in untreated outbred pigs (Cordier et al, 1966; Peacock and Terblanche, 1967; Calne et al, 1967). Sponstaneous tolerance has since been demonstrated in rats (Kamada, Brons, and Davies, 1980; Zimmerman et al, 1984) and mice (Qian et al, 1994).

Eventually, it was shown that heart (Corry, Winn, and Russell, 1973; Qian et al, 1994) and kidney allografts (Russell et al, 1978) also could self-induce donor-specific tolerance in selected mouse strain combinations. Exhaustion and deletion of an antigen-specific clone was one of the mechanisms proposed for allograft acceptance as early as 1964 and again in 1969 (Figure 1), but this was difficult to defend in the absence of chimerim. The failure to evolve an intellectual framework with which to explain organ engraftment contrasted with bone marrow transplantation in which the association of tolerance with donor leukocyte chimerism was readily apparent.

Thus, organ transplantation became disconnected from a scientific base, creating an image that was judged at times to

be dubious --- scientifically, ethically and practically. A widely expressed opinion was that chronic immunosuppression would lead inevitably to lethal infectious complications and/or the development of malignant tumors (Figure 2). These complications did, in fact, prove to be common. Infection was exemplified by the cytomegalovirus (CMV), which normally has low pathogenicity, but which has been responsible for many post-transplantation deaths as a co-infection with *Pneumocystis carinii*. De novo neoplasms, and particularly the Epstein Barr virus-associated B cell malignancies, were prototype examples of the loss of tumor surveillance (Starzl, 1969b). As it turned out, these problems were manageable.

Antilymphoid Strategies

Successful kidney transplantation was first accomplished several years before the lymphocyte had any known function, and almost a decade before the distinction between T- and B-lymphocytes was made. After Gowan's studies in rats demonstrated the defects in the immune response caused by lymphoid depletion with thoracic duct drainage (TDD) (McGregor and Gowans, 1963, 1964), TDD was used in Stockholm in 1963 and subsequently to precondition human kidney recipients (Franksson and Blomstrand, 1967). Although moderately effective, TDD was inconvenient and expensive. When Woodruff and Anderson (1963) added antilymphocyte serum (ALS) to TDD,

the effects were additive, but this combination was never used clinically.

In 1966, heterologous antilymphocyte globulin (ALG) was introduced clinically for lymphoid depletion (Starzl et al, 1967). In preclinical studies, horse anti-dog antilymphocyte serum (ALS) was raised and the active component was shown to be a gamma globulin moiety (Iwasaki et al, 1967). After demonstrating that the refined horse anti-dog ALG inhibited or reversed kidney and liver rejection in dogs, horse anti-human ALG was raised and given to human kidney recipients as a short-term adjunct to azathioprine and prednisone. After the "triple drug" therapy was shown to be effective in the kidney trial, the same treatment was used in 1967 for the first successful liver transplanatations (Starzl et al, 1968a).

Within 24 months after the first successful liver replacement, many extrarenal transplant programs (i.e. heart, lung, and pancreas) were begun, using triple drug therapy. Although isolated successes were recorded (Table 2), most of these new programs closed because of the high mortality. Nevertheless, ALG played an important role in the first successful extrarenal organ transplant procedures, and served as the therapeutic model for strategies using the more standardized antibody preparations made possibly by the hybridoma technology of Kohler and Milstein (1975).

OKT3, a monoclonal antibody directed against all T lymphocytes was introduced clinically in the early 1980s (Cosimi et al, 1981) and is still part of the immunosuppressive armamentarium. Other monoclonal antibody preparations have been developed more recently, some of which are humanized hybrids and directed at such diverse targets as T-cell subsets, adhesion molecules, and receptors for T cells or interleukin-2 (IL-2). Their diversity notwithstanding, the use of all of the monoclonal antibodies is guided by the same treatment principles that were developed with the crude ALG.

T-Cell Directed Drugs

Borel et al (1976) showed that cyclosporine depressed cellular immunity by acting with relative specificity on T lymphocytes without depressing the bone marrow and without obvious toxicity to other organs. Borel et al also reported that the new drug prolonged skin allograft survival in mice, rats, and guinea pigs. Kostakis et al (1977), Calne and White (1977), and Green and Allison (1978) then demonstrated that cyclosporine could prevent or delay heart, kidney, liver, or pancreas rejection in rats, rabbits, dogs, and pigs. After cyclosporine was introduced clinically, the dose-limiting nephrotoxicity of the drug became apparent as well as its neurotoxicity, diabetogenicity, cosmetic side effects, and propensity to induce B cell lymphomas (Calne et al, 1978,

1979). At lower doses, and in combination with prednisone (Starzl et al, 1980), the prognosis with transplantation with all kinds of organs, and especially the liver (Starzl et al, 1981), was dramatically improved (Figure 3).

Tacrolimus was first used clinically as a replacement for cyclosporine in patients who were intractably rejecting liver allografts (Starzl et al, 1989a; Fung et al, 1990). When the drug was found to rescue >75% of rejecting hepatic allografts and other kinds of rejecting organs, trials were began using tacrolimus as the baseline agent from the outset (Todo et al, 1990). Tacrolimus did not have cyclosporine's cosmetic side effects, but the nephrotoxicity, neurotoxicity, and diabetogenicity profiles were similar. As with cyclosporine, these problems were dose-related and manageable by using tacrolimus in combination with prednisone or in more complex drug cocktails. Survival of various kinds of grafts and their recipients was improved (Figure 3) and it became possible for the first time to offer intestinal transplantation as a clinical service (Todo et al, 1992). Most recently, tacrolimus has been combined with rapamycin, with unprecedented control and prevention of rejection.

ORGAN PRESERVATION

Very little research had been done on preservation of organs at the time clinical kidney transplantation suddenly

and unexpectedly became a widely used form of treatment in the early 1960s. Total body hypothermia of live donors was used initially to minimize ischemic injury to the excised kidney (Starzl et al, 1963a), but this potentially dangerous practice was promptly supplanted by infusion of chilled fluids into the allograft renal artery immediately after donor nephrectomy (Starzl, 1964b). Intravascular cooling of liver allografts with chilled lactated Ringers solution had been developed much earlier in canine liver transplant experiments and had dramatically increased the chance of survival (Starzl et al, 1960). Today, intravascular cooling derived from the in situ techniques of Marchioro et al (1963) remains the first step in the preservation of all cadaveric organs.

Two basic strategies for extending organ graft survival after initial cooling also were developed with kidneys and livers, and applied to other organs. In one, *ex vivo* perfusion techniques were used to simulate normal physiologic conditions as pioneered by Carrel and Lindberg (1938). Using blood for priming, the technology was modified by Ackerman and Barnard (1966). Because these perfusion methods were too complex for general use, Belzer, Ashby and Dunphy (1967) developed a simplified asanguinous perfusion technique, which eventually was abandoned in favor of the second option of "slush" preservation.

Slush preservation consists of intravascular infusion with chilled fluids, followed by immersion of the organ in the fluid and simple refrigeration. Collins, Bravo-Shugarman, and Terasaki (1969) replaced the original lactated Ringers solution with a perfusate that resembled the electrolyte composition of intracellular fluid. Renal allograft preservation with the "Collins solution" was reliable for at least a day, and preservation of the liver was adequate for approximately 6 hours. Nearly 20 years passed before the advent of the University of Wisconsin (UW) solution allowed the safe preservation of livers for 24 hours (Kalayoglu et al, 1988, Todo et al, 1989) with a doubling of the safe time for kidney preservation. The UW solution made the exchange of organs between different cities or countries a reality.

Before 1980, cadaveric organ procurement and kidney procurement were essentially synonymous. With the emergence of extrarenal organ transplantation, flexible techniques were developed with which the kidney, liver, heart, lung, pancreas, and even intestine could be removed separately or in combinations (Starzl et al, 1984, 1987). These flexible techniques involve cooling of all organs *in situ* and removal in a bloodless field, followed by ex vivo dissection. Taken together, the improvements in organ procurement and preservation have allowed the efficient use of donor organs,

an especially important advance in view of the world-wide shortage of essentially all kinds of allografts.

IMMUNOLOGIC SCREENING

Matching cadaveric donors against a list of prospective recipients was not possible until effective methods of organ preservation became available. Consequently, it had been predicted in the early 1960s that tissue matching and organ preservation would have to develop in parallel if long term engraftment of tissues and organs was to succeed with any degree of reliability and predictability. Instead, immunologic screening of donors and recipients played very little role in the developmental period of organ transplantation during the volatile period of 1959-1968.

The Crossmatch Principle

As it turned out, the greatest impact of pre-transplant immunologic screening has been the prevention of hyperacute rejection by observation of ABO compatibility guidelines and the routine use of the cytotoxicity crossmatch.

ABO Incompatibility --- Hyperacute rejection was first observed more than 30 years ago when ABO-mismatched renal allografts were transplanted into patients who had preformed antigraft ABO isoagglutinins (Starzl, 1964c). After such kidneys were lost on the operating table, arteriograms of the

infarcted organs showed nonfilling of the small vessels. The gross findings correlated histopathologically with widespread thrombotic occlusion of the microvasculature.

It was concluded that high-affinity ABO isoagglutinins in the recipient sera had bound to A or B antigens in the graft vessels and parenchymal cells. The guidelines formulated from this experience (Table 4) were designed to avoid such antibody confrontations (Starzl, 1964c). The ABO rules also apply to heart, liver, and other kinds of organ transplantation. However, not all organs placed in an environment made hostile by antigraft isoagglutinins meet the same fate. In addition, it was learned at an early time that the liver is more resistant to antibody attack than other organs (Starzl et al, 1974).

Preformed Antidonor Cytotoxins --- Hyperacute rejection of a kidney by an ABO compatible recipient was reported for the first time by Terasaki, Marchioro, and Starzl (1965). Terasaki's observation that the serum of the recipient of a live donor kidney contained preformed antigraft lymphocytotoxic antibodies was promptly confirmed in similar cases by Kissmeyer-Nielsen et al (1966) and by others (Williams et al, 1968; Starzl et al, 1968b). The evidence of a cause and effect relation in Terasaki's single first case was so clear that he recommended and immediately introduced

his now universally applied lymphocytotoxic crossmatch test (Terasaki, Marchioro, and Starzl, 1965; Patel and Terasaki et al, 1969).

Tissue Matching

The importance of the genetically determined major histocompatibility complex (MHC) in determining the immune response to allografts was established at a very early time by investigations in inbred mice (Gorer, Lyman, and Snell, 1948; Snell 1948). The possibility of clinical tissue matching did not begin to emerge, however, until the discovery of the first human leukocyte antigen (HLA) (Dausset, 1958), and the discovery in the same year of antileukocyte antibodies (soon shown to be HLA directed) in the sera of pregnant women (Van Rood, Eernisse, and van Leeuwen, 1958).

The report in 1964 of the microcytotoxicity test, with which HLA antigens could be detected serologically with minute quantities of sera (Terasaki and McClelland, 1964) was a further critical development in moving forward with the detection and classification of the antigens. It was anticipated that long term organ engraftment would be achievable only with a high degree of donor/recipient HLA match, and that there would be a stepwise deterioration in outcome with every level of HLA mismatch. The importance of HLA matching was immediately fulfilled with bone marrow

transplantation, in which anything less than a perfect or near perfect match between the donor and recipient resulted in GVHD or else rejection of the graft (Mathe et al, 1963; Bach et al, 1968; Gatti et al, 1968; Thomas et al, 1975).

Inexplicably at the time, Terasaki promptly recognized that kidney transplantation was not dependent on tissue matching. This was evident in a retrospective study of long surviving kidney recipients and their volunteer live donors (Starzl et al, 1965b). This was followed by a prospective trial in which kidney donors were selected on the basis of the best available HLA match for recipients who were treated with azathioprine and prednisone, with or without adjunct ALG (Terasaki et al, 1966). Although HLA matched (zero mismatched) allografts had the best survival and function, no cumulative adverse effect of mismatching in the kidney recipients could be identified (Starzl et al, 1970). This imprecise prognostic discrimination also pertained to cadaver kidney transplantation (Mickey and Terasaki, 1971) and has been evident in analyses up to the present time. The absence of a large and consistent matching effect unless there is a perfect or near perfect match has always been the same (Starzl et al, 1997). Furthermore, the difference in clinical outcome with completely matched versus variably HLA-mismatched allografts has been surprisingly small.

Terasaki's observations of the University of Colorado kidney cases nearly 3 decades ago breathed life into the still struggling fields of liver, heart, and lung transplantation. It was a relief to know that the selection of donors with random tissue matching would not result in an intolerable penalty. A quarter of a century passed before it could be explained why HLA matching was critical for bone marrow, but not organ, transplantation (see next section).

ALLOGRAFT ACCEPTANCE AND ACQUIRED TOLERANCE INVOLVE THE SAME MECHANISMS

Until the last decade, a transplanted organ was viewed as a defenseless object of immune attack by the host, subject to rejection of varying severity depending on the degree of histoincompatibility (Figure 5, upper left). In contrast, the cytoablated host after bone marrow engraftment was viewed as the defenseless object of a unidirectional graft versus host (GVH) immune reaction (Figure 5, upper right). In the early 1990s, we discovered evidence suggesting that both of these perceptions were incorrect, in that a double immune reaction occurred with both kinds of transplantation. This insight began with a study of long-term human survivors of liver, kidney and other organ transplantations.

It was discovered that all of these organ recipients had small numbers of donor leukocytes in their blood or tissue (microchimerism) (Starzl et al, 1992a, 1992b, 1993). At about the same time, it was shown that cytoablation of bone marrow recipients does not completely destroy host leukocytes (Przepiorka et al, 1991; Wessman et al, 1993) as had been previously assumed. From these findings, we concluded that a bidirectional immune reaction had taken place in both bone marrow and solid organ recipients, and that this occurred with maximum intensity in the first few post-transplant days or weeks (Figure 4). The differences between organ transplantation and bone marrow transplantation (Table 5), which had been assumed for three decades to be caused by separate mechanisms, lay instead in the relative strength of the opposing immune reactions. Furthermore, reciprocal interactions of coexisting donor and recipient leukocyte populations were necessary for alloengraftment with both kinds of transplantation (Figure 5, lower).

After organ transplantation, there is an acute migration of immunogenic multilineage "passenger" leukocytes from the graft, selectively at first to host lymphoid organs. In the meanwhile, host cells replaced most but not all of the passenger leukocytes in the graft (Figure 6). Initially, the coexisting donor and recipient cells in widespread organized lymphoid collections generate widespread host versus graft

(HVG) and graft versus host (GVH) immune activation that may proceed to reciprocal clonal exhaustion-deletion and variable degrees of donor and recipient specific non-reactivity (i.e. tolerance) (Figure 4). Maintenance of the acutely induced state require persistence of the microchimerism (Starzl and Zinkernagel, 1998b; Ehl et al, 1998; Starzl, 1998a). The same events in mirror image occur with bone marrow engraftment.

With either kind of transplantation, immunosuppression allowed the acute induction to proceed by preventing either the donor or recipient cell population from destroying the other before the clonal deletion could occur. The organs' passenger leukocytes that survive the initial immune reaction migrate secondarily to areas other than the lymphoid organs, thereby escaping attack by the host immune system (immune indifference) (Terakura et al, 1998). These "sheltered" leukocytes may then "leak" periodically to the host lymphoid organs, thus maintaining clonal exhaustion-deletion at a level compatible with allograft survival (Starzl and Zinkernagel, 1998b) (Figure 6). The greatest cell migration occurs from the leukocyte-rich liver, accounting for its unusual tolerogenicity; but the same events occur after transplantation of all organ grafts. The modulation of the host immune response by these donor cells explained the poor discrimination of HLA matching for organ transplantation.

THE IMMUNE REACTION TO INFECTIOUS MICROORGANISMS IS THE SAME AS THAT AGAINST ALLOGRAFTS AND XENOGRAFTS

In 1974-75 Zinkernagel and Doherty discovered that one of the biologic roles of the major histocompatibility complex (MHC) is the adaptive immune response directed against intracellular noncytopathic or weakly cytopathic microorganisms (Figure 7). Because the cost of total elimination of all cells infected with this kind of pathogen could be the death or invalidism of the host, a means has evolved by which the immune response can be terminated by antigen-specific clonal exhaustion-deletion, thereby allowing survival of both the microorganism and the host (Zinkernagel, 1996). Clonal deletion is governed by migration and localization of the microorganisms. In the same traffic pattern as that of migratory leukocytes, the pathogens move preferentially at first to host organized lymphoid tissues (e.g. lymph nodes, spleen) (Zinkernagel et al, 1997). After either an infection or after transplantation, host cytolytic T lymphocytes recognize the mobile antigen in a MHC-restricted context (Starzl and Zinkernagel, 1998b).

Thus, a perfect state of allograft acceptance can be compared to a continuously high load of non-cytopathic microorganisms that may lead to a pathogen-specific immunologic collapse (i.e an asymptomatic carrier state)

(Figure 7, second panel). At the other extreme, acute irreversible rejection may be compared to dramatic, efficient control of the pathogen by antigen-specific effector T cells (Figure 7, first panel). Degrees of chronic rejection of the allograft correspond to variable incomplete clonal exhaustion-deletion (Figure 7, third panel). In mirror image, the infectious disease analogy with bone marrow transplantation after pretreatment with cytoablation is infection by microorganisms that avoid migration to host lymphoid organs (e.g. rabies and wart viruses) and therefore do not induce an efficient immune response (immune indifference) (Figure 7, right).

In contrast to the MHC restricted response to noncytopathic organisms, the response to cytopathic microorganisms includes activation of the innate response effectors such as interferons, macrophages, gamma/delta T cells, natural killer (NK) cells, B cells that may continue to secrete antibodies without T cell help, early interleukins and phagocytes (Zinkernagel, 1996). The principal but probably not the only target of this uncontrollable reaction when the organs of lower mammals (e.g. of pigs) are transplanted to humans is the galactose α 1-3 galactose epitope (αGal) found in the Golgi apparatus of the cells (Galili et al, 1987; Cooper, Koren, and Oriol, 1994). If such discordant animal organs, or even those from more closely related species such

as baboons are to be transplanted clinically, it will be necessary to change the antigenic profile of the xenograft to one that is recognized by the human immune system as a non-cytopathic microorganism (i.e. comparable to an allograft). Until this is accomplished by the creation of transgenic animals, xenotransplantation will not be a viable option.

CONCLUSIONS

Over the last 40 years, progress in manipulating the mechanisms involved in the immune response has steadily increased the probability of allograft acceptance and acquired tolerance. However, the penalty has been variable weakening of the host's ability to mount an immunologic response to pathogens, to maintain tumor surveillance, or (probably) to carry out other subtle homeostatic functions. Herein lies one of the primary challenges to the future of transplantation. Hopefully, from continued study of transplantation *per se*, as well as advances in related fields, more effective solutions will be found to overcome the current barriers to allografts or even xenografts with minimum perturbation to all other facets of immune function.

SELECTED READING

Ackerman JR, Barnard CN. A report on the successful storage of kidneys. Brit J Surg 53:525-532, 1966

Anderson D, Billingham RE, Lampkin GH, Medawar PB: The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity 5:379-397, 1951.

Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM: Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet 2:1364-1366, 1968.

Barnard CN: What we have learned about heart transplants. J Thorac Cardiovasc Surg 56:457-468, 1968.

Belzer FO, Ashby BS, Dunphy JE, 24-hour and 72 hour preservation of canine kidneys. Lancet 2:536-538, 1967

Bhandari M, Tewari A: Is transplantation only 100 years old? Brit J Urol 79:495-498, 1997.

Billingham RE, Krohn PL, Medawar PB: Effect of cortisone on survival of skin homografts in rabbits. Brit Med J 1157-1163, 1951.

Billingham RE, Brent L, Medawar PB: "Actively acquired tolerance" of foreign cells. Nature 172:603-606, 1953.

Billingham R, Brent L, Medawar P: Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Philos Trans R Soc Lond (Biol) 239:357-412, 1956.

Billingham R, Brent L: A simple method for inducing tolerance of skin homografts in mice. Trans Bull 4:67-71, 1957.

Billingham R, Brent L: Quantitative studies on transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. Philos Trans R Soc Lond (Biol) 242:439-477, 1959.

Borel JF, Feurer C, Gubler HU, Stahelin H: Biological effects of cyclosporin A; a new antilymphocytic agent. Agents Actions 6:468-475, 1976.

Burnet FM, Fenner F: The Production of Antibodies. 2nd ed Melbourne, Macmillan 1949. pp: 1-142

Burnet FM: The Clonal Selection Theory of Acquired Immunity. Nashville, TN: Vanderbilt University Press, 1959. p:59

Calne RY: The rejection of renal homografts: Inhibition in dogs by 6-mercaptopurine. Lancet 1:417-418, 1960.

Calne RY (a): Inhibition of the rejection of renal homografts in dogs by purine analogues. Transplant Bull 28:445-461, 1961.

Calne RY, Murray JE (b): Inhibition of the rejection of renal homografts in dogs by Burroughs Wellcome 57-222. Surg Forum 12:118-120, 1961.

Calne RY, Alexandre GPJ, Murray JE: A study of the effects of drugs in prolonging survival of homologous renal transplants in dogs. Ann NY Acad Sci 99:743-761, 1962.

Calne RY, White HJO, Yoffa DE, Maginn RR, Binns RM, Samuel JR, Molina V: Observations of orthotopic liver transplantation in the pig. Brit Med J 2:478-480, 1967.

Calne RY, White DJG: Cyclosporin A; a powerful immunosuppressant in dogs with renal allografts. International Research Communications System Med Sci 5:595, 1977.

Calne RY, White DJG, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K: Cyclosporin A in patients

receiving renal allografts from cadaver donors. Lancet 2:1323-1327, 1978.

Calne RY, Rolles K, White DJG, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Henderson RG, Aziz S, Lewis P: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs; 32 kidneys, 2 pancreases, and 2 livers. Lancet 2:1033-1036, 1979.

Cannon JA, Longmire WP: Studies of successful skin homografts in the chicken. Ann Surg 135:60-68, 1952.

Carrel A: The operative technique for vascular anastomoses and transplantation of viscera. Lyon Medicine 98:859, 1902.

Carrel A, Lindbergh CA: The Culture of Organs. New York, P.B. Hoeber, Inc. 1938.

Collins GM, Bravo-Shugarman M, Terasaki PI. Kidney preservation for transportation: Initial perfusion and 30 hours ice storage. Lancet 2:1219-1224, 1969.

Cooper J: The evolution of techniques and indications for lung transplantation. Ann Surg 212:249-256, 1990.

Cooper DKC, Koren E, Oriol R: Oligosacchardies and discordant xenotransplantation. Immunol Rev 141:31-58, 1994.

Cordier G, Garnier H, Clot JP, Camplez P, Gorin JP, Clot Ph, Rassinier JP, Nizza M, Levy R: La greffe de foie orthotopique chez le porc. Mem Acad Chir (Paris) 92:799-807, 1966.

Corry RJ, Winn HJ, Russell PS: Primary vascularized allografts of hearts in mice: the role of H-2D, H-2K, and non-H-2 antigens in rejection. Transplantation 16:343-350, 1973.

Cosimi AB, Colvin RB, Burton RC, Rubin RH, Goldstein G, Kung PC, Hansen WP, Delmonico FL, Russell PS: Use of monoclonal antibodies to T-cell subsets for immunological monitoring and treatment in recipients of renal allografts. New Eng J Med 305:308-314, 1981.

Dausset J: Iso-leuco-anticorps. Acta Haematol 20:156-166, 1958.

Derom F, Barbier F, Ringoir S, Versieck J, Rolly G, Berzseny G, Vermeire P, Vrints L: Ten-month survival after lung homotransplantation in man. J Thorac Cardiovasc Surg 61:835-846, 1971.

Doherty PC, Zinkernagel RM. A biological role for the major histocompatibility antigens. Lancet i:1406-1409, 1975.

Dubost C, Oeconomos N, Nenna A, Milliez P: Resultats d'une tentative de greffe renale. Bull Soc Med Hop Paris 67:1372-1382, 1951.

Ehl S, Aichele P, Ramseier H, Barchet W, Hombach J, Pircher H, Hengartner H, Zinkernagel RM: Antigen persistence and time of T-cell tolerization determine the efficacy of tolerization protocols for prevention of skin graft rejection. Nature Medicine 4:1015-1019, 1998

Franksson C, Blomstrand R: Drainage of the thoracic lymph duct during homologouos kidney transplantation in man. Scand J Urol Nephrol 1:123-131, 1967.

Fung JJ, Todo S, Jain A, McCauley J, Alessiani M, Scotti C, Starzl TE: Conversion of liver allograft recipients with cyclosporine related complications from cyclosporine to FK 506. Transplant Proc 22:6-12, 1990.

Galili U, Clark MR, Shohet SB, Buehler J, Macher BA: Evolutionary relationship between the natural anti-Gal antibody and the Galα(1,3)Gal epitope in primates. Proc Natl Acad Sci (USA) 84:1369-1373, 1987.

Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA: Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet 2:1366-1369, 1968.

Goodwin WE, Kaufman JJ, Mims MM, Turner RD, Glassock R, Goldman R, Maxwell MM: Human renal transplantation. I. Clinical experience with six cases of renal homotransplantation. J Urology 89:13-24, 1963.

Gorer PA, Lyman S, Snell GD: Studies on the genetic and antigenic basis of tumour transplantation. Linkage between a histocompatibility gent and "fused" in mice. Proc Roy Soc B 135:499-505, 1948.

Goulet O, Revillon Y, Brousse N, Jan D, Canion D, Rambaus C, Cerf-Bensussan N, Buisson C, Hubert P, DePotter S, Mougenot JF, Fischer A, Ricour C: Successful small bowel transplantation in an infant. Transplantation 53:940-943, 1992.

Green CJ, Allison AC: Extensive prolongation of rabbit kidney allograft survival after short-term Cyclosporin A treatment. Lancet 1:1182-1183, 1978.

Groth CG, Brent LB, Calne RY, Dausset J, Good RA, Murray JE, Shumway NE, Schwartz RS, Starzl TE, Terasaki PI, Thomas ED, van Rood JJ: Historical landmarks in clinical transplantation: conclusions from the consensus conference held at the University of California, Los Angeles (UCLA). World J Surg, 24:834-843, 2000.

Hamburger J, Vaysse J, Crosnier J, Tubiana M, Lalanne CM, Antoine B, Auvert J, Soulier JP, Dormont J, Salmon C, Maisonnet M, Amiel JL: Transplantation of a kidney between nonmonozygotic twins after irradiation of the receiver. Good function at the fourth month. Presse Med 67:1771-1775, 1959.

Hamburger J, Vaysse J, Crosnier J, Auvert J, Lalanne CL, Hopper J, Jr.: Renal homotransplantation in man after radiation of the recipient. Am J Med 32:854-871, 1962.

Hamilton DNH, Reid WA: Yu Yu Voronoy and the first human kidney allograft. Surg Gynecol Obstet 159:289-294, 1984.

Hume DM, Merrill JP, Miller BF, Thorn GW: Experiences with renal homotransplantation in the human: report of nine cases. J Clin Invest 34:327-382, 1955.

Hume DM, Jackson BT, Zukoski CF, Lee HM, Kauffman HM, Egdahl RH: The homotransplantation of kidneys and of fetal liver and

spleen after total body irradiation. Ann Surg 152:354-373, 1960.

Iwasaki Y, Porter KA, Amend JR, Marchioro TL, Zuhlke V, Starzl TE: The preparation and testing of horse antidog and antihuman antilymphoid plasma or serum and its protein fractions. Surg Gynecol Obstet 124:1-24, 1967.

Kalayoglu M, Sollinger WH, Stratta RJ, D'Alessandro AM, Hoffman RM, Pirsch JD, Belzer FO: Extended preservation of the liver for clinical transplantation. Lancet 1(8586):617-619, 1988.

Kamada N, Brons G, Davies HffS: Fully allogeneic liver grafting in rats induces a state of systemic nonreactivity to donor transplantation antigens. Transplantation 29:429-431, 1980.

Kissmeyer-Nielsen F, Olsen S, Peterson VP, Fieldborg O: Hyperacute rejection of kidney allografts associated with preexisting humoral antibodies against donor cells. Lancet II:662-665, 1966.

Kohler G, Milstein C: Continuous culture of fused cells secreting antibody of predefined specificity. Nature 256:495-497, 1975.

Kostakis AJ, White DJG, Calne RY: Prolongation of rat heart allograft survival by cyclosporin A. International Research Communications System Med Sci 5:280, 1977.

Kuss R, Teinturier J, Milliez P: Quelques essais de greffe rein chez l'homme. Mem Acad Chir 77:755-764, 1951.

Kuss R, Legrain M, Mathe G, Nedey R, Camey M: Homologous human kidney transplantation. Experience with six patients. Postgrad Med J 38:528-531, 1962.

Lillehei RC, Simmons RL, Najarian JS, Weil R, Uchida H, Ruiz JO, Kjellstrand CM, Goetz FC: Pancreaticoduodenal allotransplantation: Experimental and clinical observations. Ann Surg 172:405-436, 1970.

Main, J.M., Prehn, R.T. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. J Natl Cancer Inst 15: 1023-1029, 1955.

Mannick JA, Lochte HL, Ashley CA, Thomas ED, Ferrebee JW: A functioning kidney homotransplant in the dog. Surgery 46:821-828, 1959.

Marchioro TL, Huntley RT, Waddell WR, Starzl TE: Extracorporeal perfusion for obtaining postmortem homografts. Surgery 54:900-911, 1963.

Mathe G, Amiel JL, Schwarzenberg L, Cattan A, Schneider M: Haematopoietic chimera in man after allogenic (homologous) bone-marrow transplantation. Brit Med J 2:1633-1635, 1963.

McGregor DD, Gowans JL: Antibody response of rats depleted of lymphocytes by chronic drainage from the thoracic duct. J Exp Med 117:303-320, 1963.

McGregor DD, Gowans JL. Survival of homografts of skin in rats depleted of lymphocytes by chronic drainage from the thoracic duct. Lancet 1:629-632, 1964.

Medawar PB: The behavior and fate of skin autografts and skin homografts in rabbits. J Anat 78:176-199, 1944.

Medawar PB: Transplantation of tissues and organs: introduction. Brit Med Bull 21:97-99, 1965.

Meeker W, Condie R, Weiner D, Varco RL, Good RA: Prolongation of skin homograft survival in rabbits by 6-mercaptopurine. Proc Soc Exp Biol Med 102:459-461, 1959.

Merrill JP, Murray JE, Harrison JH, Guild WR: Successful homotransplantation of the human kidney between identical twins. JAMA 160:277-282, 1956.

Michon L, Hamburger J, Oeconomos N, Delinotte P, Richet G, Vaysse J, Antoine B: Une tentative de transplantation renale chez l'homme. Aspects Medicaux et Biologiques. Presse Med 61:1419-1423, 1953.

Mickey MR, Kreisler M, Albert ED, Tanaka N, Terasaki PI: Analysis of HL-A incompatibility in human renal transplants. Tissue Antigens 1:57-67, 1971.

Murray JE, Merrill JP, Harrison JH: Renal Homotransplantation in identical twins. Surg Forum 6:432-436, 1955.

Murray JE, Merrill JP, Dammin GJ, Dealy JB, Jr., Walter CW, Brooke MS, Wilson RE: Study of transplantation immunity after total body irradiation: Clinical and experimental investigation. Surgery 48:272-284, 1960.

Murray JE, Merrill JP, Dammin GJ, Dealy JB, Jr., Alexandre GW, Harrison JH: Kidney transplantation in modified recipients. Ann Surg 156:337-355, 1962.

Murray JE, Merrill JP, Harrison JH, Wilson RE, Dammin GJ: Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. New Engl J Med 268:1315-1323, 1963.

Murray JE, Sheil AGR, Moseley R, Knoght PR, McGavic JD, Dammin GJ: Analysis of mechanism of immunosuppressive drugs in renal homotransplantation. Ann Surg 160:449-473, 1964.

Murray JE, personal communication, March 27, 1999.

Owen RD: Immunogenetic consequences of vascular anastomoses between bovine twins. Science 102:400-401, 1945.

Patel R, Terasaki PI: Significance of the positive crossmatch test in kidney transplantation. New Eng J Med 280:735-739, 1969.

Peacock JH, Terblanche J: Orthotopic homotransplantation of the liver in the pig. In: Read AE, ed. The Liver. London: Butterworth. 1967. pg 333.

Pierce JC, Varco RL: Induction of tolerance to a canine renal homotransplant with 6-mercaptopurine. Lancet I:781-782, 1962.

Przepiorka D, Thomas ED, Durham DM, Fisher L: Use of a probe to repeat sequence of the Y chromosome for detection of host cells in peripheral blood of bone marrow transplant recipients. Am J Clin Pathol 95:201-206, 1991.

Qian S, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE: Murine liver allograft transplantation: Tolerance and donor cell chimerism. Hepatology 19:916-924, 1994.

Reemstsma K. McCracken BH, Schlegel JU, Pearl MA, Pearce CW, DeWitt CW, Smith PE, Hewitt RL, Flnner RL, Creech O Jr.: Renal heterotransplantation in man. Ann Surg 160:384-410, 1964.

Russell PS, Chase CM, Colvin RB, Plate JMD: Kidney transplants in mice. An analysis of the immune status of mice bearing long-term H-2 incompatible transplants. J Exp Med 147:1449-1468, 1978.

Schwartz R, Dameshek W: The effects of 6-mercaptopurine on homograft reactions. J Clin Invest 39:952-958, 1960.

Servelle M, Soulie P, Rougeulle J: Greffe d'une rein de supplicie a une malade avec rein unique congenital, atteinte de nephrite chronique hypertensive azatemique. Bull Soc Med Hop Paris 67:99-104, 1951.

Simonsen M: The impact on the developing embryo and newborn animal of adult homologous cells. Acta Path Microbiol Scand 40:480-500, 1957.

Snell GD: Methods for the study of histocompatibility genes. J Genet 49:87-103, 1948.

Starzl TE, Kaupp HA Jr, Brock DR, Lazarus RE, Johnson RV: Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 111:733-743, 1960.

Starzl TE, Brittain RS, Stonnington OG, Coppinger WR, Waddell WR (a): Renal transplantation in identical twins. Arch Surg 86:600-607, 1963.

Starzl TE, Marchioro TL, Waddell WR (b): The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet 117:385-395, 1963.

Starzl TE (a): Experience in Renal Transplantation. W.B. Saunders Company, Philadelphia, 1964, pp 166-169; 360-362.

Starzl TE (b): Experience in Renal Transplantation. W.B. Saunders Company, Philadelphia, 1964, pp 68-71.

Starzl TE (c): Patterns of Permissible Donor-Recipient Tissue Transfer in Relation to ABO Blood Groups. In: Experience in Renal Transplantation. Philadelphia, PA Saunders, 1964, pp 37-47.

Starzl TE, Marchioro TL, Peters GN, Kirkpatrick CH, Wilson WEC, Porter KA, Rifkind D, Ogden DA, Hitchcock CR, Waddell WR (d): Renal heterotransplantation from baboon to man: Experience with 6 cases. Transplantation 2:752-776, 1964.

Starzl TE, Marchioro TL, Porter KA, Taylor PD, Faris TD, Herrmann TJ, Hlad CJ, Waddell WR (a): Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog. Surgery 58:131-155, 1965.

Starzl TE, Marchioro TL, Terasaki PI, Porter KA, Faris TD, Herrmann TJ, Vredevoe DL, Hutt MP, Ogden DA, Waddell WR (b): Chronic survival after human renal homotransplantations: Lymphocyte-antigen matching, pathology and influence of thymectomy. Ann Surg 162:749-787, 1965.

Starzl TE, Marchioro TL, Porter KA, Iwasaki Y, Cerilli GJ: The use of heterologous antilymphoid agents in canine renal

and liver homotransplantation and in human renal homotransplantation. Surg Gynecol Obstet 124:301-318, 1967.

Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ Jr, Porter KA (a): Orthotopic homotransplantation of the human liver. Ann Surg 168:392-415, 1968.

Starzl TE, Lerner RA, Dixon FJ, Groth CG, Brettschneider L, Terasaki PI (b): Shwartzman reaction after human renal transplantation. N Engl J Med 278:642-648, 1968.

Starzl TE (a): Efforts to Migigate or Prevent Rejection. In: Experience in Hepatic Transplantion. W.B. Saunders Company, Philadelphia, PA. 1969. pp: 228-233

Starzl, TE: (b): Late results and complications. In: Experience in Hepatic Transplantation. WB Saunders Company, Philadelphia 1969. pp: 348-390.

Starzl TE, Porter KA, Andres G, Halgrimson CG, Hurwitz R, Giles G, Terasaki PI, Penn I, Schroter GT, Lilly J, Starkie SJ, Putnam CW: Long-term survival after renal transplantation in humans: With special reference to histocompatibility matching, thymectomy, homograft glomerulonephritis,

heterologous ALG, and recipient malignancy. Ann Surg 172:437-472, 1970.

Starzl TE, Ishikawa M, Putnam CW, Porter KA, Picache R, Husberg BS, Halgrimson CG, Schroter G: Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. Transplant Proc 6:129-139, 1974.

Starzl TE, Weil R III, Iwatsuki S, Klintmalm G, Schroter GPJ, Koep LJ, Iwaki Y, Terasaki PI, Porter KA: The use of cyclosporin A and prednisone in cadaver kidney transplantation. Surg Gynecol Obstet 151:17-26, 1980.

Starzl TE, Klintmalm GBG, Porter KA, Iwatsuki S, Schroter GPJ: Liver transplantation with use of cyclosporin A and prednisone. N Engl J Med 305:266-269, 1981.

Starzl TE, Hakala TR, Shaw BW Jr, Hardesty RL, Rosenthal TE, Griffith BP, Iwatsuki S, Bahnson HT: A flexible procedure for multiple cadaveric organ procurment. Surg Gynecol Obstet 158:223-230, 1984.

Starzl TE, Miller C, Broznick B, Makowka L: An improved technique for multiple organ harvesting. Surg Gynecol Obstet 165:343-348, 1987.

Starzl TE, Rowe M, Todo S, Jaffe R, Tzakis A, Hoffman A, Esquivel C, Porter K, Venkataramanan R, Makowka L, Duquesnoy R (a): Transplantation of multiple abdominal viscera. JAMA 261:1449-1457, 1989.

Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramanan R, Jain A (b): FK 506 for human liver, kidney and pancreas transplantation. Lancet 2:1000-1004, 1989.

Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M (a): Cell migration, chimerism, and graft acceptance. Lancet 339:1579-1582, 1992.

Starzl TE, Demetris AJ, Trucco M, Ramos H, Zeevi A, Rudert WA, Kocova M, Ricordi C, Ildstad S, Murase N (b): Systemic chimerism in human female recipients of male livers. Lancet 340:876-877, 1992.

Starzl TE, Demetris AJ, Trucco M, Murase N, Ricordi C, Ildstad S, Ramos H, Todo S, Tzakis A, Fung JJ, Nalesnik M, Zeevi A, Rudert WA, Kocova M: Cell migration and chimerism after whole-organ transplantation: The basis of graft acceptance. Hepatology 17:1127-1152, 1993.

Starzl TE, Eliasziw M, Gjertson M, Terasaki PI, Fung JJ, Trucco M, Martell J, McMichael J, Shapiro R, Donner A: HLA and cross reactive antigen group (CREG) matching for cadaver kidney allocation. Transplantation 64:983-991, 1997.

Starzl TE (a): The art of tolerance. Nature Medicine 4: 1006-1009, 1998.

Starzl TE, Zinkernagel RM (b): Antigen localization and migration in immunity and tolerance. New Eng J Med 339:1905-1913, 1998.

Starzl TE: History of Clinical Transplantation. World J Surg, 24:759-782, 2000.

Terakura M, Murase N, Demetris AJ, Ye Q, Thomson A, Starzl TE: Lymphoid/non-lymphoid compartmentalization of donor leukocyte chimerism in rat recipients of heart allografts, with or without adjunct bone marrow. Transplantation 66:350-357, 1998.

Terasaki PI, McClelland JD: Microdroplet assay of human serum cytoxins. Nature 204:998-1000, 1964.

Terasaki PI, Marchioro TL, Starzl TE: Sero-typing of human lymphocyte antigens: Preliminary trials on long-term kidney

homograft survivors. In: Histocompatibility Testing National Acad Sci-National Res Council, Washington, DC, 1965, pp. 83-96.

Terasaki PI, Vredevoe DL, Mickey MR, Porter KA, Marchioro TL, Faris TD, Starzl TE: Serotyping for homotransplantation. VI. Selection of kidney donors for thirty-two recipients. Ann NY Acad Sci 129:500-520, 1966.

Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, Lerner KG, Glucksberg H, Buckner CD: Bone-marrow transplantation. N Engl J Med 292:832-843, 895, 1975.

Todo S, Nery J, Yanaga K, Podesta L, Gordon RD, Starzl TE: Extended preservation of human liver grafts with UW solution. JAMA 261:711-714, 1989.

Todo S, Fung JJ, Starzl TE, Tzakis A, Demetris AJ, Kormos R, Jain A, Alessiani M, Takaya S: Liver, kidney, and thoracic organ transplantation under FK 506. Ann Surg 212:295-305, 1990.

Todo S, Tzakis AG, Abu-Elmagd K, Reyes J, Nakamura K, Casavilla A, Selby R, Nour BM, Wright H, Fung JJ, Demetris AJ, Van Thiel DH, Starzl TE: Intestinal transplantation in

composite visceral grafts or alone. Ann Surg 216:223-234, 1992.

Trentin JJ: Mortality and skin transplantibility in X-irradiated mice receiving isologous or heterologous bone marrow. Proc Soc Exper Biol Med 92:688-693, 1956.

Van Rood JJ, Eernisse JG, van Leeuwen A: Leucocyte antibodies in sera of pregnant women. Nature 181:1735-1736, 1958.

Voronoy U: Sobre bloqueo del aparato reticuloendotelial del hombre en algunas formas de intoxicacion por el sublimado y sobre la transplantacion del rinon cadaverico como metodo de tratamiento de la anuria consecutiva a aquella intoxicacion. (Blocking the reticuloendothelial system in man in some forms of mercuric chloride intoxication and the transplantation of the cadaver kidney as a method of treatment for the anuria resulting from the intoxication.) Siglo Medico 97:296-297, 1937.

Wessman M, Popp S, Ruutu T, Volin L, Cremer T, Knuutila S: Detection of residual host cells after bone marrow transplantation using non-isotopic in situ hyubridization and karyotype analysis. Bone Marrow Transplant 11:279-284, 1993.

Willimas GM, Hume DM, Hudson RP, Morris PJ, Kano K, Milgrom F: "Hyperacute" renal-homograft rejection in man. New Eng J Med 279:611-618, 1968.

Woodruff MFA, Anderson NF: Effect of lymphocyte depletion by thoracic duct fistula and administration of anti-lymphocytic serum on the survival of skin homografts in rats. Nature (London) 200:702, 1963.

Zimmerman FA, Davies HS, Knoll PP, Gocke JM, Schmidt T: Orthotopic liver allografts in the rat. Transplantation 37:406-410, 1984.

Zinkernagel RM, Doherty PC: Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choiromeningitis within a syngeneic or semi-allogeneic system. Nature 248:701-702, 1974.

Zinkernagel RM. Immunology taught by viruses. Science 271:173-178, 1996.

Zinkernagel RM, Ehl S, Aichele P, Oehen S, Kundig T, Hengartner H. Antigen localization regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. Immunol Reviews 156:199-209, 1997.

Zukoski CF, Lee HM, Hume DM: The prolongation of functional survival of canine renal homografts by 6-mercaptopurine. Surg Forum 11:470-472, 1960.

Zukoski CF, Callaway JM: Adult tolerance induced by 6-methyl mercaptopurine to canine renal homografts. Nature (London) 198:706-707, 1963.

TABLE 1: NOBEL PRIZES RELATED TO IMMUNOLOGY/TRANSPLANTATION
(Source: Nobel Foundation, Stockholm)

Year	Name	Accomplishment
1901	Emil Adolf Von Behring	Discovery of antibodies.
1905	Heinrich Hermann Robert Koch	Cause and effect of microorganisms and infection.
1908	Ilya Metchnikoff	Champion of cellular immunity.
	Paul Ehrlich	Side chain (receptor) concept; antimicrobial therapy. Champion of humoral immunity;
1912	Alexis Carrel	Vascular surgery and transplantation.
1919	Jules Bordet	Discovery of complement.
1930	Karl Landsteiner	Discovered ABO blood group antigens.
1960	Sir Frank MacFarlane Burnet	Clonal selection hypothesis.
	Sir Peter Brian Medawar	Acquired transplantation tolerance.
1972	Gerald M. Edelman	Characterized immunoglobulins.
	Rodney R. Porter	Clarified structure of antibody molecule.
1980	Baruj Benacerrat	Discovered immune response genes
	Jean Dausset	Discovered first HLA antigen.
	George Davis Snell	Discovery of major histocompatibility complex (MHC) in mice.
1984	Niels Kaj Jerne	Important immunologic hypotheses.
	Georges J.F. Kohler	Hybridoma technology.
	Cesar Milstein	Hybridoma technology.
1985	Michael Stuart Brown	Hepatic control of cholesterol metabolism
	Joseph Leonard Goldstein	
1987	Susumu Tonegawa	Discovered somatic recombination of immunologic receptor genes.
1988	Gertrude Belle Elion	Co-discoveredof 6-MP and azathioprine.
	George Herbert Hitchings	
1990	Joseph E. Murray	Kidney transplantation.
	E. Donnall Thomas	Bone marrow transplantation.
1996	Rolf Zinkernagel	Co-discovered the role of MHC restriction
	Peter C. Doherty	in adaptive immune response to pathogens.

TABLE 2: FIRST SUCCESSFUL TRANSPLANTATION OF *

HUMAN ALLOGRAFTS (SURVIVAL > 6 MONTHS)

ORGAN	CITY	DATE	PHYSICIAN/SURGEON (DATE PUBLISHED)
Kidney	Boston	1/24/59	Murray et al (1960)
Bone Marrow	Paris	4/23/63	Mathe et al (1963)
Liver	Denver	7/23/67	Starzl et al (1968)
Heart	Cape Town	1/2/68	Barnard (1968)
Pancreas*	Minneapolis	6/3/69	Lillehei et al (1970)
Lung**	Ghent	11/14/68	Derom et al (1971)
Abdominal multivisceral***	Pittsburgh	11/1/87	Starzl et al (1989)
Intestine alone	Paris	3/18/89	Goulet et al (1992)

*Kidney and pancreas allografts in uremic patient.

**Patient died after 10 months. The first > one year survival of isolated lung recipient was not reported until 1987 (Cooper et al 1990).

***Small and large bowel plus liver, pancreas, stomach, and duodenum. The patient died after 6 months.

TABLE 3

KIDNEY TRANSPLANTATION

≥ 6 MONTHS SURVIVAL AS OF MARCH 1963

Case	City	Date	Donor	Survival (months)+
1.	Boston	1-24-59	Frat twin	>50
2.	Paris	6-29-59	Frat twin	>45
3.	Paris	6-22-60	Unrelated*	18 (Died)
4.	Paris	12-19-60	Mother*	12 (Died)
5.	Paris	3-12-61	Unrelated*	18 (Died)
6.	Paris	2-12-62	Cousin*	>13
7.	Boston	4-5-62	Unrelated	10

*Adjunct steroid therapy

+The kidneys in patients 1, 2, and 6 functioned for 20.5, 25, and 15 years respectively. Patient 7 rejected his graft after 17 months and died 7 months after return to dialysis.

Cases 1 and 7: Murray et al (1960, 1963)

Cases 2, 4, and 6: Hamburger et al (1959, 1962)

Cases 3 and 5: Kuss et al (1962)

TABLE 4

DIRECTION OF ACCEPTABLE ORGAN TRANSFER WHEN THE DONOR AND RECIPIENT HAVE DIFFERENT ABO RED CELL TYPES* (Starzl, 1964c)

O to non-O	Safe
Rh- to Rh+	Safe
Rh+ to Rh-	Relatively safe
A to non-A	Dangerous
B to non-B	Dangerous
AB to non-AB	Dangerous

*For organ transplantation, O is universal donor and AB is universal recipient. With the transplantation of bone marrow allografts, or of lymphoid rich organ allografts (e.g. intestine or liver), enough antihost isoagglutinins may be produced by the allograft to cause serious or lethal hemolysis in a significant number of cases (humoral GVHD). Consequently, the rules summarized in this table are fully applicable only with leukocyte-poor organs like the kidney and heart.

TABLE 5

DIFFERENCES BETWEEN CONVENTIONAL BONE MARROW

AND ORGAN TRANSPLANTATION (Starzl and Zinkernagel 1998b)

Bone Marrow				**Organ**
Yes	<--	Recipient Cytoablation*	-->	No
Critical	<--	MHC Compatibility	-->	Not Critical
GVHD	<--	Principal Complication	-->	Rejection
Common	<--	Drug Free State	-->	Rare
Tolerance	<--	Term for Success	-->	"Acceptance"**

*Note: All differences derive from this therapeutic step which in effect establishes an unopposed GVH reaction in the bone marrow recipient whose countervailing immune reaction is eliminated.

**Or "operational tolerance"

FIGURE LEGENDS

Figure 1 --- Hypothesis published in 1969 of allograft acceptance by clonal exhaustion. Antigen presentation was depicted via the macrophages rather than by the dendritic cells (which had not yet been described). A gap in this hypothesis was the failure to stipulate the location of the immune activation (Starzl 1969a)

Figure 2 --- The historic concern that there would be simultaneous loss of host ractivity to specific strains of endogenous bacteria, as well as to the alien renal tissue.

Figure 3 --- The 3 eras of orthotopic liver transplantation at the Universities of Colorado (1963-80) and Pittsburgh (1981-1993), defined by azathioprine (AZA), cyclosporine (CYA), and tacrolimus/(TAC)-based immune suppression. Patient survival was about 10% higher than graft survival in both the cyclosporine (1980-89) and tacrolimus eras (1989-93) because of effective hepatic retransplantation, an option that did not exist previously. Similar stepwise improvements were seen with transplantation of all organs.

Figure 4 --- Contemporaneous host versus graft (HVG) and graft versus host (GVH) reactions in the two-way paradigm of transplantation immunology. Following the initial interaction leading to clonal exhaustion-deletion, maintenance of the

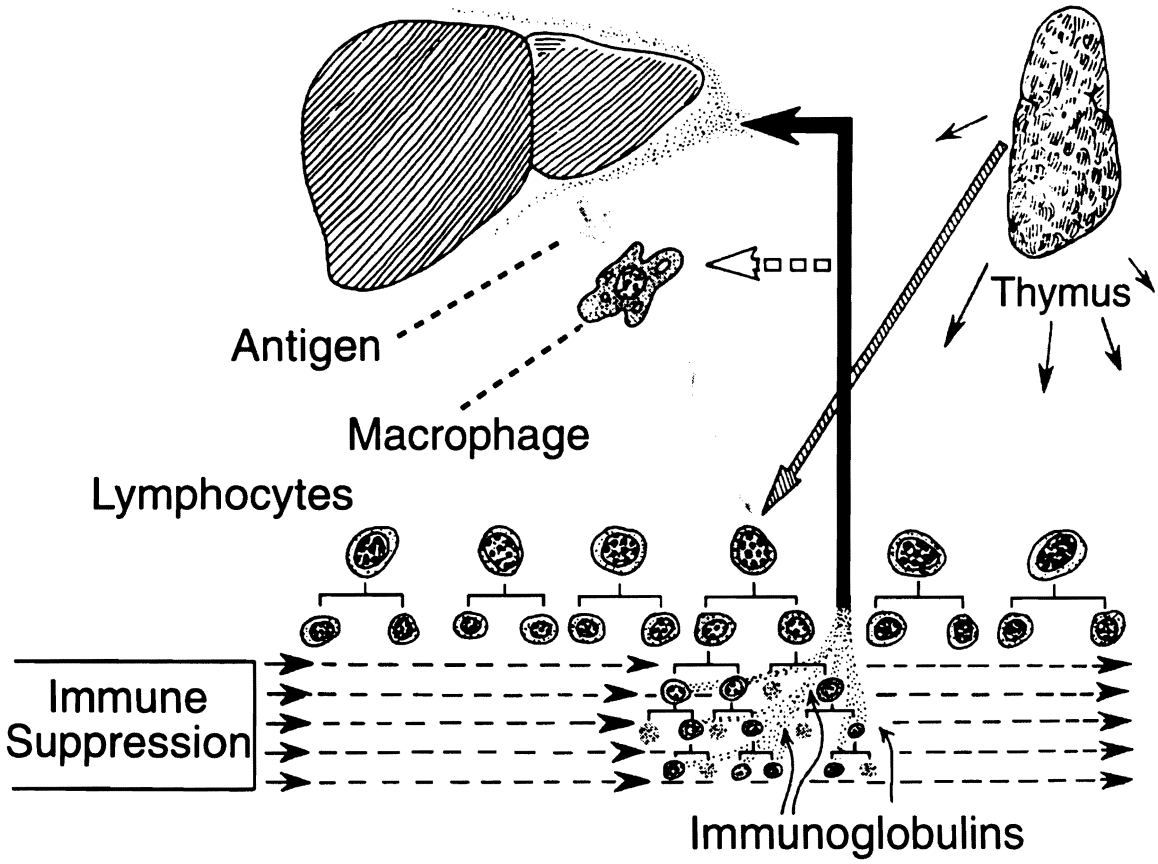
exhaustion-deletion state is dependent on persistent donor leukocyte chimerism (see text and Figure 6). Thus, continued allograft acceptance/tolerance is depicted as a low-grade stimulatory state.

Figure 5 --- (T**op panels**) One-way paradigm in which transplantation is conceived as involving a unidirectional immune reaction: (**left**) host-versus-graft (HVG) with whole organs and (**right**) graft-versus-host (GVH) with bone marrow or other lymphopoietic transplants. (B**ottom panels**) Two-way paradigm with which transplantation is seen as a bidirectional and mutually cancelling immune reaction that is (**left**) predominantly HVG with whole organ grafts, and (**right**) predominantly GVH with bone marrow grafts.

Figure 6 --- The four events that occur in close temporal approximation when there is successful organ engraftment. Double acute clonal exhaustion (1,2). Maintenance of the clonal exhaustion-deletion requires persistence of donor leukocytes in recipient tissues (3) and in the allograft (4) The "accepted " allograft is never completely depleted of these donor cells and it serves as a preferential site for donor stem cells.

Figure 7 --- Variable outcomes after infection with widely disseminated non-cytopathic viruses (or other

microorganisms) and analogies (text below the panels) to organ and bone marrow transplantation. The horizontal axis denotes time, and the vertical axis shows the viral load (blue line), and the host immune response (dashed red line).



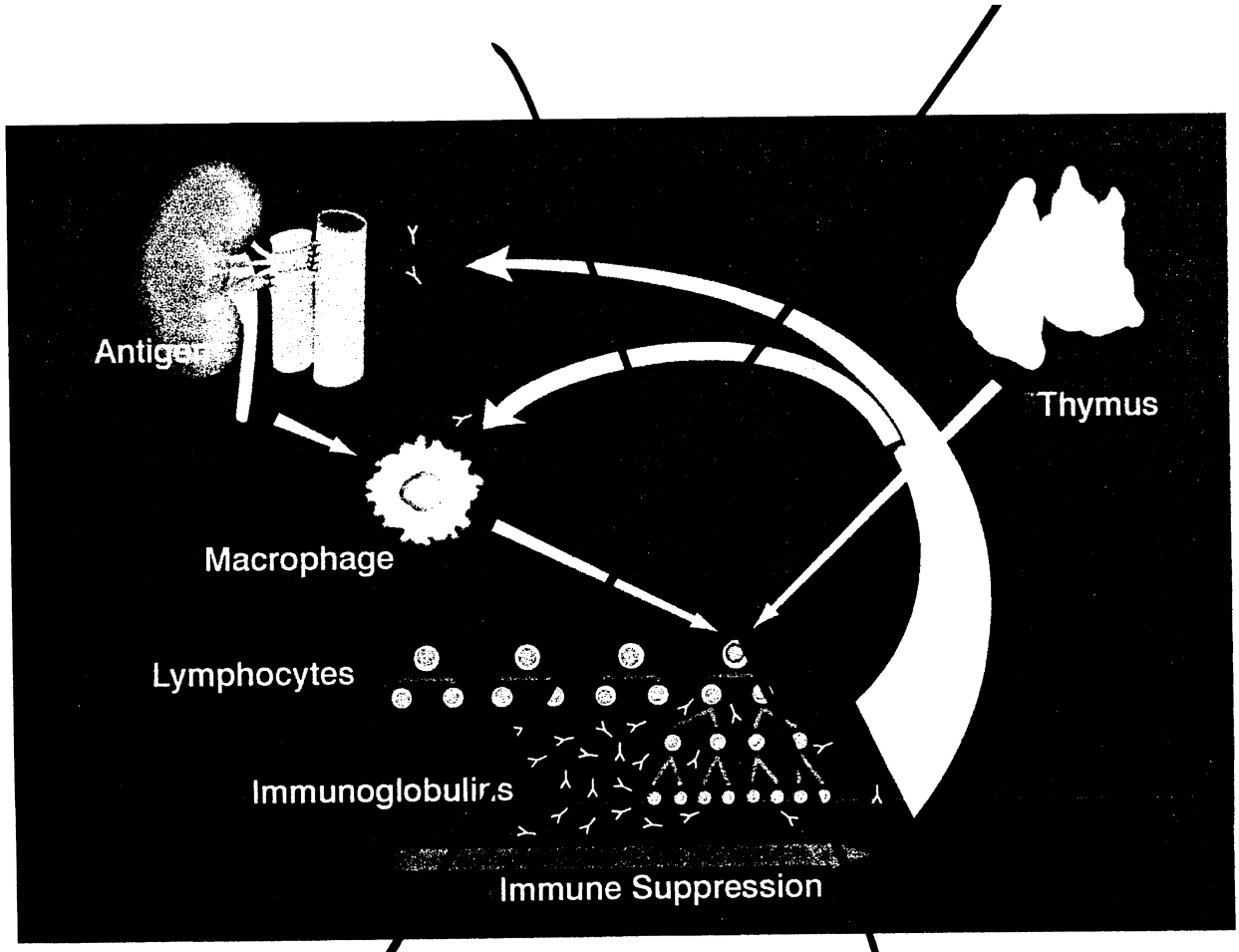


Figure 1
Hypothesis published in 1969 of allograft acceptance by clonal exhaustion.

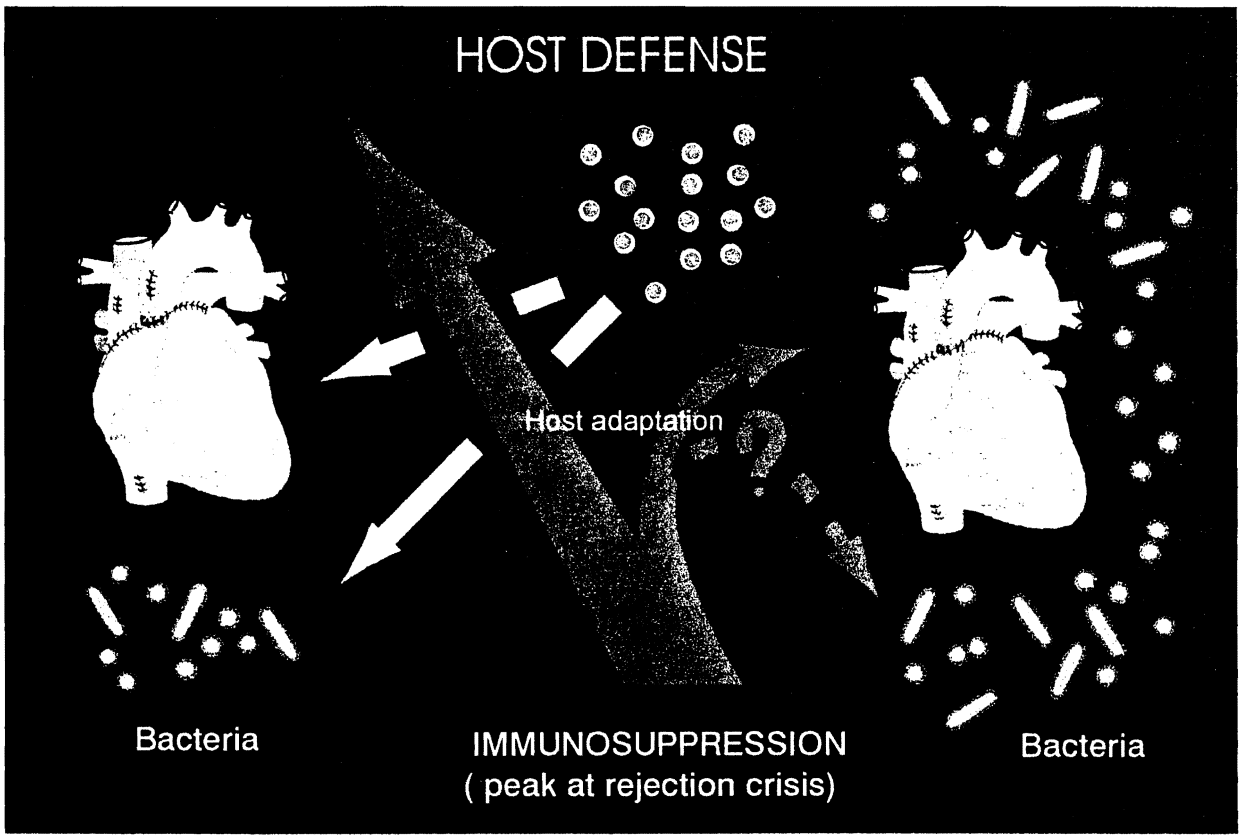


Figure 2
Possible mechanisms of simultaneous loss of host reactivity to specific strains of endogenous bacteria, as well as to the alien renal tissue.

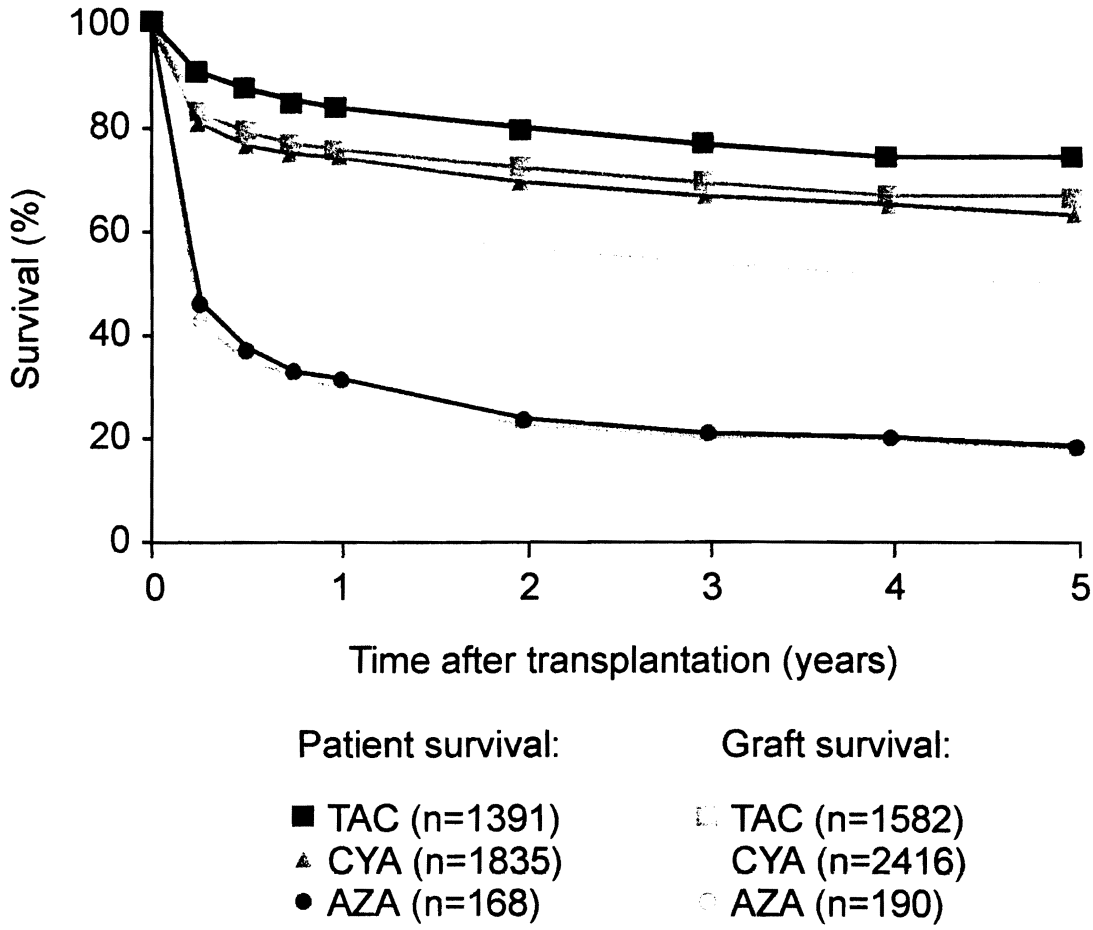


Figure 3

The 3 eras of orthotopic liver transplantation at the Universities of Colorado (1963-80) and Pittsburgh (1981-1993), defined by azathioprine/(AZA), ciclosporin/(CYA), and tacrolimus/(TAC)-based immune suppression. The same stepwise improvements were seen in all organs. Here there was about 10% lower than patient survival in both the ciclosporin (1980-88) and tacrolimus eras (1989-93) because of effective retransplantation, an option that did not exist previously.

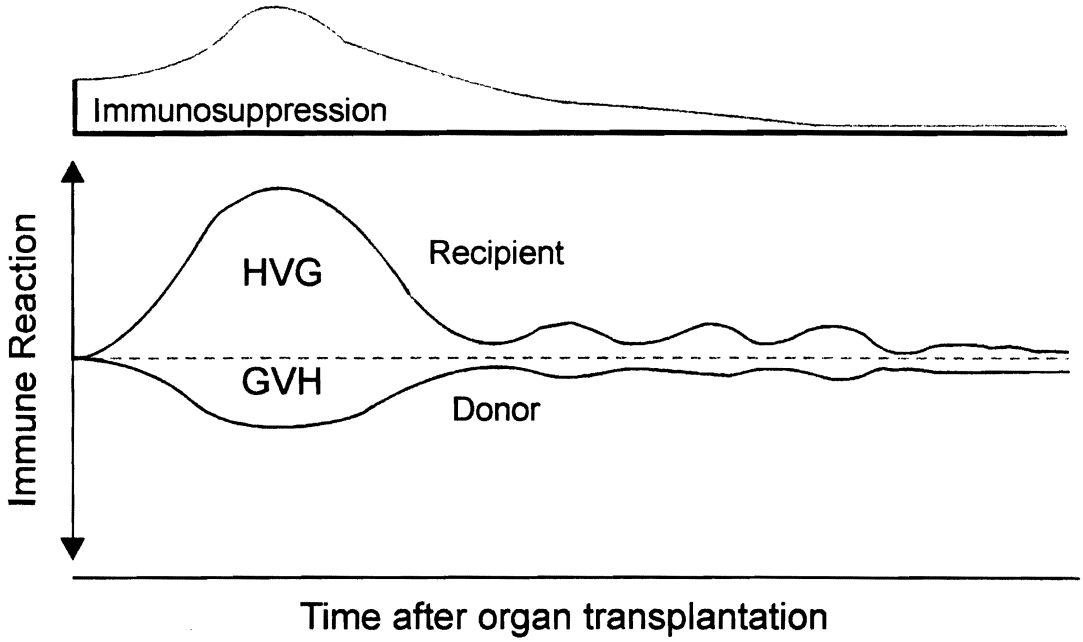


Figure 4

Contemporaneous host versus graft (HVG) and graft versus host (GVH) reactions in the two-way paradigm of transplantation immunology. Following the initial interaction ~~the evolution of non-reactivity of each leukocyte population to the other is seen as a predominantly low-grade stimulatory state that may wax and wane, rather than a deletional one.~~ ~~complete tolerance~~ ~~reversible deletion.~~

Maintenance of the exhaustion-deletion state is dependent on persistent donor leukocyte Chimerism (see Text). Thus allograft acceptance/tolerance is depicted as a ↑ (and Figure 6)

leading to Clonal exhaustion-deletion

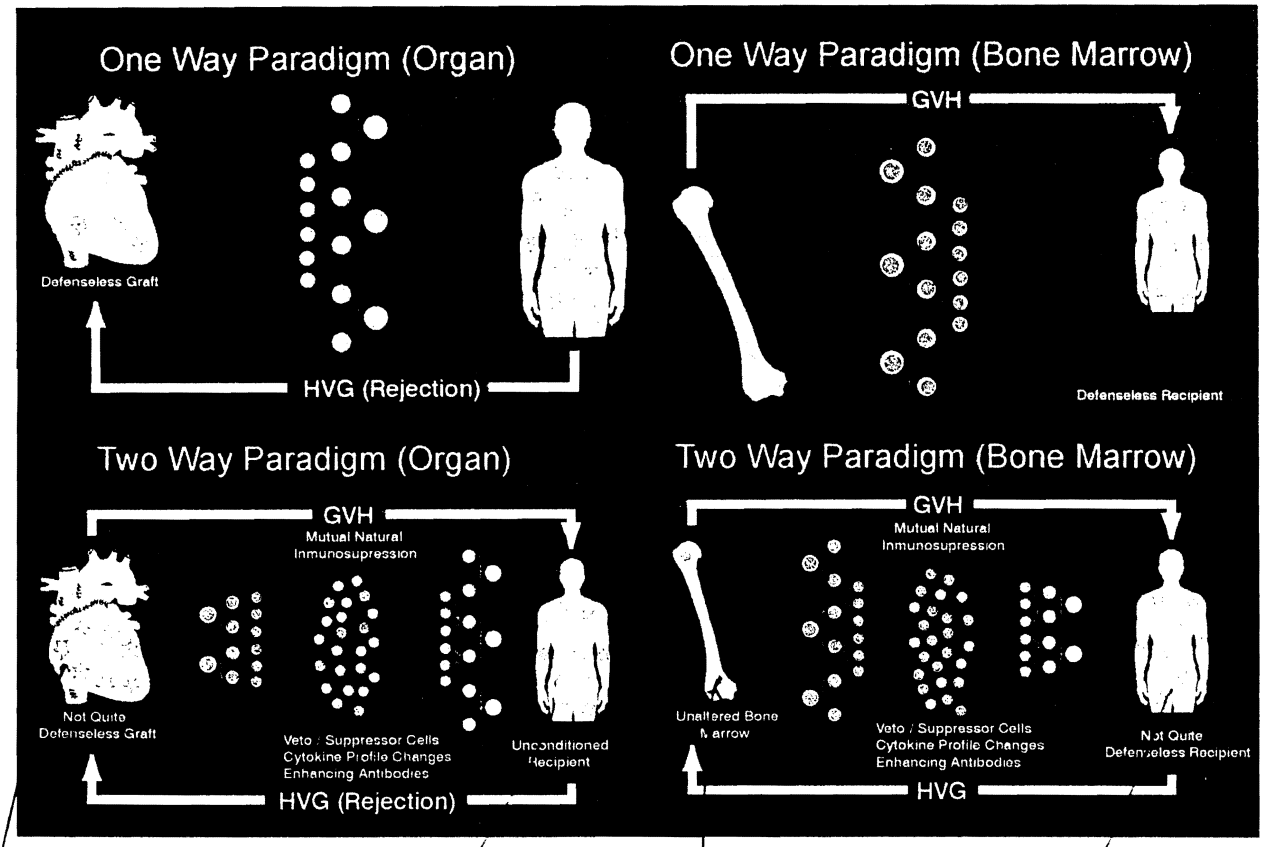


Figure 5
(top panels)
One-way paradigm in which transplantation is conceived as involving an unidirectional immune reaction: **(left)** host-versus-graft (HVG) with whole organs and **(right)** graft-versus-host (GVH) with bone marrow or other lymphopoietic transplants.
(bottom panels)
Two-way paradigm with which transplantation is seen as a bidirectional and mutually canceling immune reaction that is **(left)** predominantly HVG with whole organ grafts, or **(right)** predominantly GVH with bone marrow grafts.

should have small number of donor cells

should have small number of recipient cells

attention

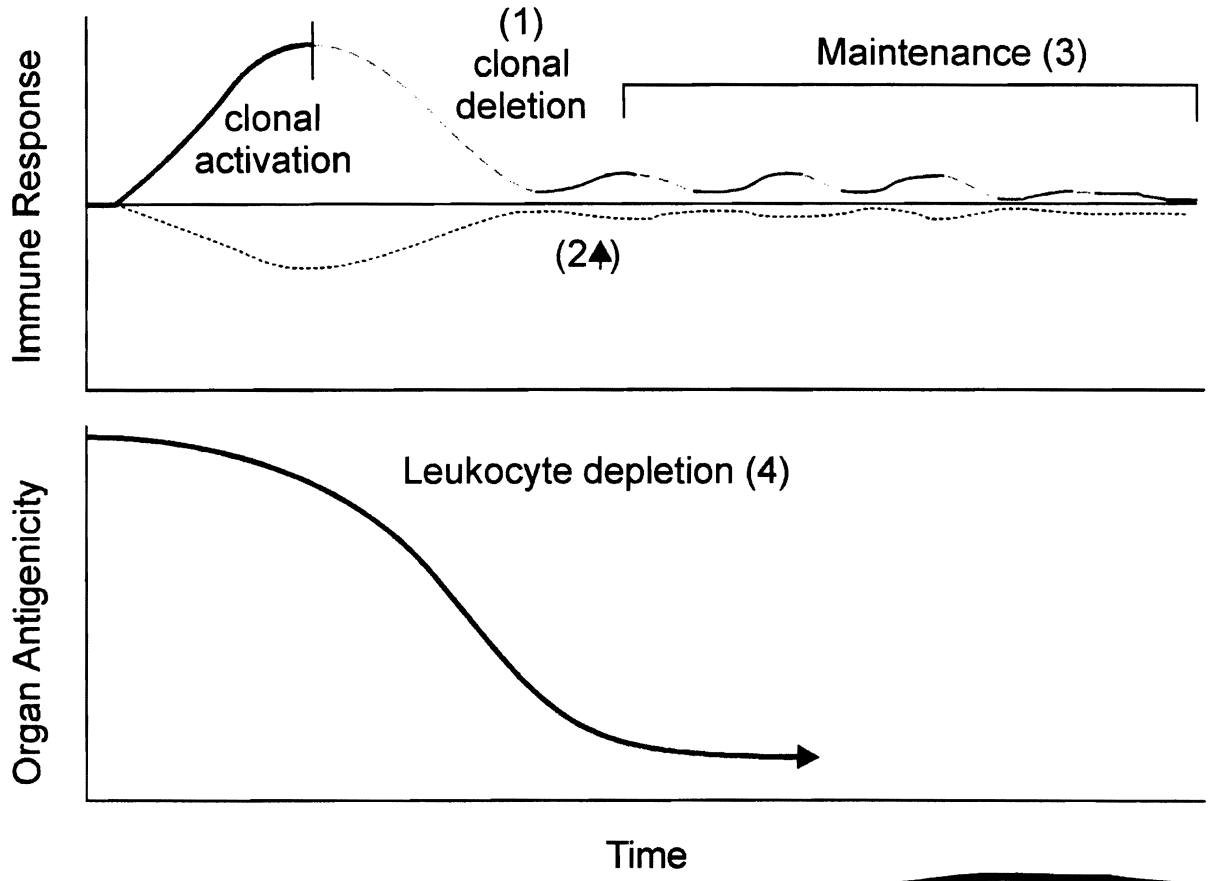


Figure 6

The ~~four events~~ that occur in close temporal approximation when there is successful organ engraftment: above, double ~~acute~~ clonal exhaustion (~~2~~) ~~slug~~ below, ~~loss~~ of ~~organ immunogenicity due to depletion of the graft's passenger leukocytes (4).~~ Note that

(1, 2 ✗)

→ Maintenance of clonal exhaustion-deletion requires persistence of donor leukocytes in recipient tissues (3) and if the allograft ~~is~~ is never completely depleted of these donor cells, the ~~allograft~~ serves as a reservoir for donor cells

Stem

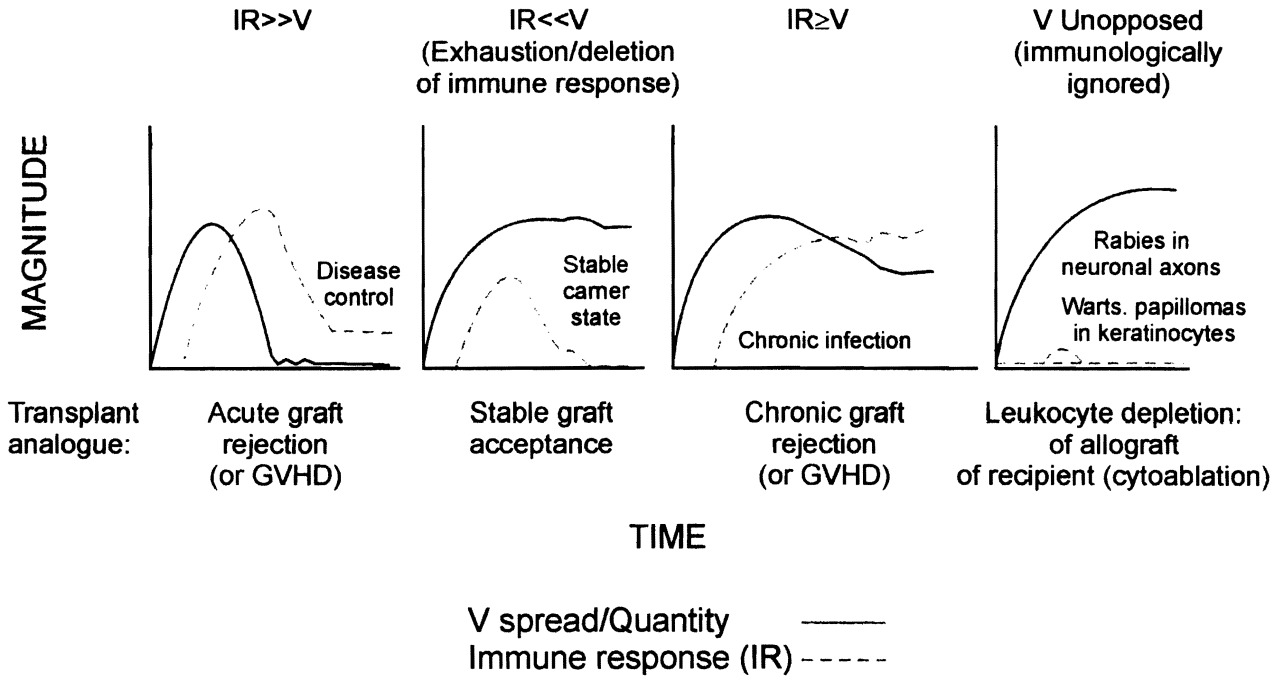


Figure 7

Variable outcomes after infection with widely disseminated non-cytopathic viruses (or other pathogens) and analogies (~~in the subscripts~~) to organ and bone marrow transplantation. The horizontal axis denotes time, and the vertical axis shows the viral load (blue line), and the host immune response (dashed red line).