



# Transplantation Timeline

## MANKIND'S THREE MILLENNIA— ONE MAVERICK'S THREE DECADES IN THE STRUGGLE AGAINST BIOCHEMICAL INDIVIDUALITY

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### Mythic Timeline

In the transplantation timeline that spans three millennia, organ replacement begins as the medicine of mythology. How better treat a diseased or injured tissue or organ than replace it completely? Chimeric gods and heroes appear in a number of cultures. Probably the first and most famous is Ganesha, the god of wisdom and vanquisher of obstacles, a Kumar child upon whom the Hindu god Shiva xenografted an elephant head. This twelfth century B.C. Aryan legend in the *Rig-Veda* was written during the Western eras of the Hebrew Exodus from Egypt and the Trojan War. Six centuries later (just after the time of Homer and during the life of Confucius) Ezekiel in the Old Testament alluded to transplantation: "A new heart also will I give you, and a new spirit will I put within you; and I will take away the stony heart out of your flesh, and I will give you a heart of flesh." In addition to deities, legendary doctors performed transplants. Pien Ch'iao, who was born about 430 B.C., corresponding to the lifetime of Socrates, sought to replace superstition with a practice of medicine founded on rational principles. He used four methods of diagnosis—examining the face, listening to the respiration, taking the history, and checking the pulse. The writings of Lieh Tzu narrated that Pien Ch'iao treated Ch'i Ying, who displayed a strong spirit but a weak will, and Kung He, in whom the opposite was true, by an exchange of their hearts to cure the unbalanced equilibrium of the two men's energies. In the West in the fourth century A.D., at the time of the Byzantine era, the twin brothers Saints Cosmas and Damian traveled through Asia Minor healing without reward and eventually dying as martyrs during Diocletian's persecution of Christians. The classic *Leggenda Aurea* of

Jacopo da Varagine recalls the “miracle of the black leg” believed to have occurred in 348 A.D. The lower extremity of a recently buried Ethiopian Moor gladiator was retrieved from the Hill of St. Peter to replace the gangrenous limb of Deacon Justinian, the sacristan of the Roman basilica that was later dedicated to the saints. This is the first recorded use of cadaver donor tissue for transplantation.

## Surgery Timeline

The second century B.C. skill of the Indian surgeon Sushruta using skin autografts for rhinoplasty was rediscovered in the first century A.D. Greek text *De Medicina*. Gaspare Tagliacozzi, a sixteenth-century Italian surgeon who restored lost noses with autografts, seemed to be the first physician to appreciate biochemical individuality: “The singular character of the individual entirely dissuades us from attempting this work (tissue transplantation) on another person.” Three centuries later, John Converse reasoned that Tagliacozzi appreciated the barrier to allotransplantation. In contradistinction, John Hunter’s successful engraftment of a human tooth into a cock’s comb in the eighteenth century led him to believe that “transplantation is founded on a disposition in all living substances to unite when brought into contact with each other.” Although Baronio in 1804 claimed successful grafting of both autogenous and xenogeneic skin transplants in sheep, Paul Bert disputed this finding in his 1863 thesis “*De la Greffe Animale*,” which described his own animal experiments using skin and tissue, allo-, xeno-, and rat parabiont “Siamese” grafts.

The last quarter of the nineteenth century witnessed advances in suturing techniques by Jaboulay, Murphy, and Payr. In 1902, Emerich Ullman of Vienna autotransplanted a dog’s kidney to the nuchal vessels and attempted allografts as well as xenografts. Floresco in Bucharest successfully transplanted saline-perfused donor kidneys into nephrectomized hosts using ureteroureteral anastomoses. By 1910, Unger claimed over 100 successful experimental kidney transplants from fox terriers to boxer dogs; Zaaier reported long-term success in canine kidney autotransplants. Using unique vascular techniques, Jaboulay of Lyon tested pig and goat kidney transplants in man. One of his assistants, Alexis Carrel, collaborated with Guthrie to perfect the triangulation vascular anastomosis technique using continuous silk suture mounted on fine needles. On the one hand, Guthrie transplanted a complete dog head onto the neck of another dog. On the other hand, Carrel performed experimental transplants of vessels, kidneys, thyroid, parathyroid, heart, ovary, and limbs; developed the internal vascular shunt; reported aortic patching with inert foreign substances; and cultivated adult tissues and organs outside the body. In recognition of his multiple contributions, he won the 1912 Nobel Prize in Physiology and Medicine. As one century earlier Baron Boyer had boasted that all major problems in surgery had been solved, so did Carrel in 1914 suggest that little work remained to perfect transplantation techniques. Although short-term survivals of corneal transplants reported in 1872 by Power were followed by consistent success in the reports of Filatov in 1924 and Castroveijo in 1931, the first human cadaver donor kidney allograft by the Ukrainian surgeon Yu. Yu. Voronoy in 1933 was unsuccessful, as were his five subsequent attempts during the next 13 years.



In 1947 Hume achieved transient function of a human kidney allograft anastomosed *ex vivo* to the vessels of the arm of a woman, a procedure that had been a technical failure in the hands of Ullman. Fortunately she spontaneously recovered from postpartum acute renal failure. While an orthotopic cadaver kidney transplant into a woman with polycystic kidney disease was probably technically unsuccessful, the Parisian surgeons Kuss, Servelle, and Dubost developed successful heterotopic techniques. However, all of the allografts eventually failed due to the lack of immunosuppression, although one living related donor graft functioned 22 days. Joe Murray, an honorary member of this society, has reported nine cases in which the hemodialysis technique designed by Willem Kolff was performed under the direction of John Merrill for preoperative preparation of end-stage renal disease patients. But these—as well as similar attempts reported by surgeons from Cleveland, London, and Los Angeles—produced only temporary function.

Exploiting the scientific foundations laid by Little and Tyzzer and the success of Bauer with skin grafts, Joe Murray documented permanent survival of an identical twin donor kidney transplant in December 1954 as well as six more cases during the next four years; approximately 30 isografts had been done worldwide by fall 1963. Thereafter some of the greatest technical advances in twentieth-century surgery were described by members of our society, including transplantation of the heart by Lower and Shumway, lung by Hardy, liver by Starzl, pancreas by Lillehei, and heart-lung by Reitz. Even eight decades after Carrell's boasting, members of this society continue to explore technical challenges in transplantation. However, translation of these surgical feats to acceptable long-term clinical results demands methods to prevent graft rejection.

### Immunology Timeline

The oriental practice of variolation for prevention of smallpox originated about 1000 A.D. In 1798 Jenner rediscovered this technique for cowpox vaccination, initiating the modern era of immunology. By 1888, the year of the dedication of the Pasteur Institute, immunologists had reported major advances: identification of many human bacterial pathogens; development of Pasteur vaccination for chicken cholera, anthrax, and rabies; and formulation of Metchnikoff's phagocytic theory of host resistance. Nuttall's experiments on serum bactericidins and Roux and Yersin's on diphtheria toxin documented natural immunity to be mediated by antigen-antibody complexes. Ehrlich's postulate that natural antibodies represent shed cell surface receptors for important nutrients suggested that antigens were substances that mimicked nutrient ligands. In the productive final decade of the nineteenth century, Koch demonstrated hypersensitivity to the tubercle bacillus; his students von Behring and Kitasato, the therapeutic potential of antitoxins; Bordet and Gengou, complement activity; Pfeiffer and Kolle, immune bacteriolysis; Von Gruber and Durham, bacterial agglutination; Belfanti and Carbone, immune hemolysis; and Kraus, precipitin reactions.

At the turn of the twentieth century, immunologists continued their prolific output: in 1899 Metchnikoff reported the activity of antilymphocyte serum; in 1900



Landsteiner discovered the ABO blood groups; in 1902 Portier and Richet documented anaphylaxis; in 1903, the Arthus reaction; in 1904 Donath and Landsteiner, the first autoimmune disease; and in 1906 both Obermeyer and Pick, the immunologic specificity of reactions to chemically modified antigens, and Von Pirquet and Shick, serum sickness. Shortly thereafter, Landsteiner's classic study *The Specificity of Serological Reactions*, introduced the concept of hapten inhibition; Prausnitz and Kustner, the passive transfer of allergy by humoral antibody; and Dienes and Schoenheit, delayed hypersensitivity to simple proteins.

## Transplantation Immunology

Onto this stage of burgeoning knowledge came seminal developments in transplantation immunology. Little and Tyzzer used the methods of Mendelian genetics in Japanese Waltzing mice to document inheritance of factors eliciting host resistance. Subsequent elegant analyses by Snell utilized congenic techniques to document a codominant major histocompatibility gene complex that controls transplant survival between inbred mouse strains.

The mechanism by which these genetic differences caused graft rejection was uncertain. In 1903, Jensen suggested that active immunity destroyed foreign tumor grafts, a concept affirmed by Schone's term "transplantationsimmunitat" with which Lexer concurred. Both Bashford et al. in 1908 and Russell in 1912 observed accelerated rejection of murine skin grafts, providing a scientific basis for the immunity theory. Davis in 1917, Shawan in 1919, and Williamson in 1923 suggested that the biological incompatibility between donor and recipient was due to disparate blood groups. In 1924, Holman reported that the "anaphylactic hypersensitivity" induced by skin allografts was specifically directed toward repeat donor but not third-party tissue transplants.

An alternate hypothesis of local graft rejection emerged in contradistinction to the systemic immunity theory. Ehrlich's 1906 "athrepsia" theory used a nutritional basis to explain the results of zig-zag transplants between allogeneic and autologous hosts: after eight days of residence on an allogeneic host, grafts were only viable if transplanted back to their original donors. These findings were interpreted to document that after eight days grafts require a fresh nutrient supply only provided by the "self" environment. Because Leo Loeb failed to use the same donor for experiments testing the survival of repeat grafts, he never observed accelerated rejection and thus espoused a local rejection theory based upon "individuality differentials." A foreign host's chemistry failed to provide the proper "fit" (or nutrient environment) complementary to the unique template of donor tissue. Upon this stage of controversy came Peter Medawar's carefully designed, stringently controlled experiments. Kindled by Tom Gibson's observations that repeat donor skin grafts in humans were rejected more quickly than the initial ones, Medawar (89, 90) documented in rabbits that transplantation induces systemic, specific "active immunization."

Although as early as 1910 DaFano observed large numbers of lymphocytes in rejected allografts, in 1951 Arnold Rich could still say: "There are numerous



reasons . . . for believing that lymphocytes play a role of importance in acquired resistance, though the precise manner in which they act is still obscure, chiefly because so little is known about the function of these cells.” In contradistinction, rapid advances in the understanding of antibodies began with Woglom’s documentation of humoral mediators of tumor resistance in 1933, followed by chemical characterization of these unique serum constituents by Tiselius et al. Within two decades antibodies were quantitated by Coons’ fluorescence, and Kabat’s hapten inhibition methods. Porter and Edelman described the heavy- and light-chain structure of antibody molecules; Kunkel et al., their uniquely reactive sites, idiotypes; Jerne, idiotypic-anti-idiotypic immunoregulation; and Tonegawa and colleagues, gene arrangements producing immunoglobulin specificity. The importance of humoral components of the host response was underscored by Williams and Hume and their colleagues, as well as by Kissmeyer-Nielsen et al., who described the destructive effects of preformed cytotoxic antibodies on allografts. While crossmatching techniques to detect cytotoxic antibodies have significantly reduced the incidence of this most pernicious hyperacute rejection, humoral mediators of acute and chronic vascular injuries remain unclear.

In addition, molecular understanding of cell-mediated resistance, a prime mover in allorejection, remains incomplete. The transfer of delayed hypersensitivity by immune cells was demonstrated in 1945 by Chase, who recognized the need for inbred animals to avert allorejection of the adoptive effectors. Using similar methods, cells were shown to carry immunologic memory toward foreign tumor grafts by Mitchison and toward allografts by Billingham et al. The critical role of the thymus was described by J.F.A.P. Miller: thymectomy of newborn animals prevented the development of cellular immunity, and lymphocyte repopulation following irradiation required the presence of the thymus. Thus, the mediators of cellular resistance became known as T cells because of their mandatory maturation in the thymus.

Although distinctive surface antigens on different lymphocyte subpopulations were postulated to explain the inconsistent reactivities of polyclonal antilymphocyte sera, identification and isolation of T cell subsets required development of a panel of monoclonal antibodies by Kung et al., using Kohler and Milstein’s hybridoma technology. A physiologic relation between T cell subsets was proposed in the two-signal hypothesis of Lafferty and Cunningham: a first humoral signal generated by antigen-presenting elements to helper-inducer T cells is transduced as a second humoral signal from these cells to effector T (and B) elements. However, time has eroded an absolute correlation between CD4 or CD8 surface phenotype and T cell functional activities—namely, T and B cell collaboration, production of lymphokine humoral mediators, and direct target killing. Indeed, the mechanisms of T cell triggering, transducing, and effecting cellular resistance remain important current research objectives.

### **Molecular Basis of Alloimmunity**

*The histocompatibility antigens.* During the past three decades, chemical techniques have elucidated the molecular basis of biologic individuality. The human major histo-



compatibility complex (HLA), including class I, class II, and other ill-defined loci encoding antigenic products, spans 3.5 million DNA base pairs—2% of the genetic material in autosomal chromosome 6. Class I and class II glycoproteins serve as scaffolds for presentation of antigens in accord with the T cell restriction hypothesis of Zinkernagel and Doherty: T cells recognize foreign markers only when presented with histocompatibility antigen. This function explains the relation of these glycoproteins to immunoresponsiveness originally noted by McDevitt and Tyan. Second, and possibly coincident to their first function, these markers trigger alloimmunity. Initial hypotheses of a lipid or carbohydrate nature of the antigenic epitopes that determine transplantation polymorphism were disproved by the demonstration that the immunogenic materials extracted with sonic energy display the buoyant density of protein. Transplantation polymorphism is due to peptide sequence differences: glycoprotein antigens purified from histoincompatible hosts displayed distinctive amino acid compositions and unique peptides on two-dimensional maps.

Class I (HLA-A,B,C) genes encode polymorphic heavy peptide chains noncovalently bound to an invariant 99 amino acid  $\beta_2$  microglobulin stipulated by chromosome 15. Although class I products are normally expressed on all nucleated cells to present antigenic peptides to CD8 cells, thereby triggering cytotoxic activity, these antigens are not essential for survival. Transmembrane class I glycoprotein heavy chains include distinctive extracellular, intramembrane, and intracellular portions. In contradistinction to the regions of conserved amino acid sequences, namely the TcR binding sites of the  $\alpha_1$  and the CD8 coreceptor site of the  $\alpha_3$  domains, papain-extracted HLA-A2 antigens show polymorphic regions in the extracellular  $\alpha_1$  and  $\alpha_2$  domains. The  $\alpha_1$  residues 9-74 and  $\alpha_2$  residues 95-156 each display 8  $\alpha$ -helical turns and 4  $\beta$ -pleated sheets forming a peptide-binding cleft. Antigenic recognition pockets along the cleft bind side chains or ends of peptides at  $\alpha_1$  positions 74 (residues 74, 97, 116) and 45 (residues 24, 26, 34, 45, 67). Peptide binding "instructs" the class I histocompatibility antigen to fold into a conformation necessary to bind  $\beta_2$ m. Indeed most surface antigen bears bound peptide, even when it is a "self"-constituent—namely, modestly polymorphic products of an MHC-linked gene, or, more probably, incidental "stand-ins." Malinger and Bevan propose that the high frequency of alloreactive CD8-positive T cells includes immune elements recognizing transplantation polymorphism plus those reactive toward the associated cleft-borne, foreign- or self-peptide antigen. The allospecific polymorphism corresponds to the peptide binding sites of the  $\alpha_1$  and  $\alpha_2$  domains in a "boomerang" distribution—namely, running along the inner edges of the helices and subadjacent  $\beta$  sheets of a variety of class I A, B, and C markers, suggesting that this biochemical individuality confers species variation for the recognition of foreign peptides. Class II (HLA-DR, DP, DQ) and 13 molecular chains each bear one peptide-binding and one immunoglobulin domain in three-dimensional motifs similar to those of class I antigens. The  $\alpha_1$  and  $\beta_1$  domains on the outer surface and sides of the antigen-presenting cleft trigger CD4 cells, thereby inducing and amplifying the immune response.

The assembly of histocompatibility antigen-peptide complexes is vulnerable to chemical manipulation. Class I (and, to a lesser extent, class II) antigenic markers uti-



lize an endogenous assembly pathway. A chaperonin-like protein, possibly the heat-shock protein HSP70, transfers peptides, which have been either degraded within or experimentally introduced into the cytoplasm, to class I markers synthesized in the endoplasmic reticulum. Antigenic structure determines the efficiency of this process: Townsend found that influenza virus nucleoprotein molecules were assembled more readily after modification of the N-terminal amino acid. Detailed structure-function correlations have emerged from binding analyses using mutant HLA-A2 molecules with pure virus peptides. In the next step, class I peptide complexes are transported from the endoplasmic reticulum to the Golgi apparatus, a phase vulnerable to Brefeldin A. On the other hand, an exogenous assembly pathway links class II antigens to antigenic peptides generated after endocytosis and digestion of antigen in endosomes by cathepsins B and D, a process sensitive to weak bases such as chloroquine. The class II MHC markers synthesized intracellularly are associated with, but not inactivated by, an invariant chain, which is proteolytically cleaved after peptide association and before surface expression.

One strategy to avoid rejection seeks to match the polymorphic transplantation antigens present on donor and recipient. Human leukocyte antigen (HLA) typing defines these determinants with polyclonal antibodies. However, this system shows extensive crossreactivity attributed to the sharing by different antigens of "public" (modestly polymorphic) epitopes. In addition, modern chemical techniques reveal that even the purportedly distinctive HLA antigens display micropolymorphism—for example, six subtypes are easily distinguished among patients bearing HLA-B27. Thus recipients of "the same" HLA-type as the allograft donor are not identical matches, but only part of an antigenic family, the members of which can discriminate foreign epitopes within common determinants on each other's tissues. Thus the HLA system oversimplifies biochemical individuality. The statement by Fuller et al. that "matching in organ transplantation tells us little about the true degree of compatibility" has been amply confirmed in clinical practice. Although monoclonal antibodies and DNA sequencing techniques may permit "epitope matching" of HLA subgroups, the tremendous polymorphism makes "perfect matches" even more unlikely, as Medawar concluded three decades ago. Of greater social concern is the likelihood that a shift to epitope matching will aggravate the Caucasian preference of the present system, since subgroups of the HLA markers commonly represented in minority groups are poorly understood. Alternatively, the failure of the existent HLA system to assure transplant success may be due to the contribution(s) of at least some of the other 35 genes in the major histocompatibility complex, including HLA-E, F, and G.

*The T cell receptor, the second major component.* The two forms of TcR include  $\alpha/\beta$  dimers on the majority of mature peripheral blood T cells, and  $\gamma/\delta$  dimers on a smaller number of lymphocytes, many of which do not appear to be classical T cells. The  $\alpha\beta\gamma\delta$  polypeptide chains share similar 110 amino acid sequences that are characteristic of the immunoglobulin superfamily and intrinsically complementary to HLA antigens. The chains bear extracellular membrane-distal variable, and membrane-proximal constant, domains that are anchored via transmembrane portions to short cytoplasmic tails. TcR diversity is generated by three mechanisms: germline variation,



somatic mutation, and a strictly regulated, site-specific, recombination process that links distinct variable, diversity, and joining gene segments. A single, shared recombinase, which is found only in immature B and T cells, assembles unique structures by randomly rearranging TcR variable region genes. The recombinase complex performs three steps: cleaving a gene segment flanked by a specific recognition sequence using a site-specific endonucleolytic mechanism, catalyzing nucleotide addition or removal at the DNA ends, and ligating the modified segments.

The binding of peptide-histocompatibility antigen assembly to clonotypic TcR is solidified by three sets of independent, accessory receptor/ligand interactions: CD8 markers to class I or CD4 to class II MHC molecules; leukocyte function associated antigen-1 (LFA-1) to intercellular adhesion molecules ICAM-1 or ICAM-2, two members of the immunoglobulin supergene family; and CD2 (LFA-2), the sheep erythrocyte receptor, to LFA-3.

*Alloimmune signal transduction.* There is only fragmentary biochemical knowledge concerning the cytoplasmic pathways that transduce the membrane signal. Foreign transplantation antigen binding to host TcR induces CD3 complex perturbation via noncovalent salt bridges. The CD3 complex, which contains five ( $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\eta$ ,  $\zeta$ ) peptide products of duplicated immunoglobulin genes, displays two motifs, each linked to a distinct T lymphocyte activation pathway: 90% as  $\gamma$ ,  $\delta$ ,  $\epsilon$  chains with  $\zeta$ ,  $\zeta$  homodimers; the rest as  $\gamma$ ,  $\delta$ ,  $\epsilon$  chains with  $\eta$ - $\zeta$  heterodimers. Ligand-occupied  $\eta$ - $\zeta$  receptors trigger a GTP-dependent protein that activates phosphoinositol phospholipase C. This enzyme catalyzes hydrolysis of phospho-inositol-diphosphate ( $\text{PIP}_2$ ) to inositol 1, 4, 5-triphosphate, which releases  $\text{Ca}^{2+}$  from intracellular endoplasmic reticulum storage sites (in the fashion of calcium ionophores), and to diacylglycerol (DAG), which activates protein kinase C (PKC), in the fashion of phorbol esters. This pathway, which may be affected by the CD-5 surface marker, produces multiple secondary effects: generating arachidonic acid as a lipoxygenase substrate, opening voltage-insensitive  $\text{Ca}^{2+}$  channels, and activating PKC catalysis of phosphorylation, including CD3  $\lambda$  and  $\delta$  chains.

In contrast,  $\zeta$ - $\zeta$  homodimers couple membrane events to the T cell-specific, tyrosine protein kinase, isozyme  $\text{pp56}^{\text{lck}}$ . Once the CD4 or CD8 coreceptor crosslinks to invariant  $\alpha 3$  domains of histocompatibility antigens,  $\text{pp56}^{\text{lck}}$  at the inner surface of the plasma membrane is dephosphorylated by the cytoplasmic tail of the CD45 (T200) membrane-bound phosphatase. Then  $\text{pp56}^{\text{lck}}$  is autophosphorylated at a different position to generate the active enzyme that phosphorylates the CD3  $\zeta$  chain, thereby triggering a poorly understood cytoplasmic pathway that is independent of  $\text{PIP}_2$ , DAG, and PKC.

TcR-CD3 complex activation is generally accompanied by, but not exclusively dependent upon, increased intracellular  $\text{Ca}^{2+}$ , which is triggered, for example, by Interleukin-1 and/or Interleukin-6. The calcium signal, which by itself is tolerogenic, stimulates DAG and PKC species distinct from the  $\eta\zeta$  pathway, as well as  $\text{Ca}^{2+}$ , calmodulin-, or cyclic nucleotide-dependent, protein kinases and phospholipase C activities. Treatment with a combination of calcium ionophore plus PKC activator/tumor promoter mimics the alloantigen signal. Additional surface markers



are crosslinked during TcR-CD3 complex activation in mature lymphocytes—namely, the CD45 phosphatase, and a nonpolymorphic 50 kDa CD2 protein. TcR/CD3 membrane triggered calcium-dependent activation events are also linked to rotarase enzymes that alter protein conformation from extended to globular structures, including *cis-trans* peptidyl-prolyl isomerases.

Nuclear activation after TcR/CD3 stimulation depends upon the appearance of inducible enhancer binding proteins, resultant from enzymatic generation, conformational changes, or protein synthesis. These regulatory proteins cooperatively bind enhancer sites on DNA, thereby attracting RNA polymerase II activity, which is necessary for *de novo* transcription of over 70 gene products associated with the T cell response. The majority of these genes are cycloheximide resistant—namely, their transcription does not depend upon preliminary protein synthesis; in contradistinction, the lesser group of cyclohexamide-sensitive, protein synthesis-dependent genes act at the later stages immediately prior to or following the initiation of cell division. The earliest antigen-induced (cyclosporine-resistant) genes during the  $G_0$ - $G_1$  transition of T cell activation are *c-fos*, which encodes a 55 kDa nuclear DNA-binding phosphoprotein Fos, and *c-myc*, which produces a nuclear protein involved in DNA synthesis and critical for entry into S phase. The *c-jun* protooncogene is up-regulated upon IL-1 stimulation to express a 39 kDa regulator, activation protein -1 (AP-1), which binds Fos to form one of a system of four transcriptional regulators of IL-2 synthesis. These enhancers, which lie 5' to the IL-2 gene, probably represent paradigms of regulatory motifs controlling other critical growth events. One CsA-sensitive regulatory protein, nuclear factor of activated T cells (NFAT-1), is generated after TcR-CD3 (but not PKC/phorbol ester) triggering and requires protein and RNA synthesis. The other two enhancer sites include the ubiquitous octamer binding protein, Oct-1 and NF- $\kappa$ B (see below).

Alternate activation pathways mediated by cytokine receptors or CD28 surface markers provide signals qualitatively different from TcR-CD3 stimulation. The CD28 pathway is an alternative to CD2-linkage that uniquely activates a GMP-dependent, CsA-resistant, protein kinase enhancing lymphokine mRNA transcription. Among the cytokine pathways, the best understood involves IL-2/IL-2R. Individual activation stimuli, including PKC, calcium ionophores, antigen-MHC, anti-TcR-CD3 antibodies, or IL-2/IL-2R $\beta$  (p75) chain binding induce IL-2R $\alpha$  (p55) chain transcription to assemble highly avid  $\alpha/\beta$  chain IL-2R complexes. A major regulatory protein of this pathway is nuclear factor- $\kappa$ B (NF- $\kappa$ B), which is similar to the enhancer controlling constitutive expression of Ig  $\kappa$  light chain genes in B cells. After phosphorylation of its inhibitory protein by PKC, NF- $\kappa$ B is activated, dimerized, and translocated to the nucleus to bind homologous DNA sequence elements. IL-2R $\alpha$  chain expression is also controlled by multiple enhancers. IL-2R-mediated protein events, which occur during the  $G_1$  phase and are CsA-resistant, include IL-2 internalization and binding to the nucleus, protein kinase activation, and up-regulated lipoxygenase activity. IL-2R triggering leads to transcription of *c-myc*, and uniquely of *c-myb*, but not of *c-fos*, protooncogenes. Another cytokine IL-6 acts synergistically with phytohemagglutinin lectin



stimulation and independent of IL-2 and PKC to activate lymphocytes. Inhibition of the IL-2 and IL-6 pathways represents a unique mode of action of rapamycin.

Both TcR-CD3 and cytokine stimulation pathways increase ornithine decarboxylase transcription during  $G_1$ . This activity represents the rate limiting factor for the synthesis of the polyamines putrescine, spermidine, and spermine-organic cations required for many growth-related functions of nucleic acid and protein synthesis. Improved understanding of histocompatibility antigen surface recognition systems and intermediate intracellular signal transduction pathways inducing gene expression should open new horizons to sabotage allorejection.

### **Immunosuppression Timeline**

The four stages of the immunosuppression timeline parallel developments in immunology. The first stage, which spanned seven decades, harnessed radiation or chemical agents to nonselectively destroy all rapidly dividing cells. In 1908, Benjamin and Sluka documented that total-body irradiation impairs the capacity of rabbits to produce precipitating antibodies toward bovine serum. In 1914, Murphy showed irradiation mitigated the development of immunity toward tumor allografts. In 1915, Hektoen concluded that lymphocytes produce antibodies, since irradiation both depleted lymphoid structures and impaired humoral immune responses. The unique radiosensitivity of lymphocytes was confirmed by the Taliaferros in 1951. Total-body irradiation prolonged canine renal allograft survival in the work of Mannick et al. as well as of Rapaport et al., and yielded 9/25 patients with successful human kidney transplants beyond two years in Hamburger's series. Although the total-lymphoid irradiation method of Kaplan as applied in renal transplantation by Strober and colleagues and the wide field method of Myburgh have refined the technique, most transplant practitioners think that this modality displays a particularly "slippery slope" of immunosuppression—namely, a propensity toward not-infrequent, slowly reversible, and rarely predictable toxic side effects accompanied by a high mortality from infection.

The pharmacologic era of immunosuppression began in 1914 when Murphy — and, two years later, Hektoen—documented the effects of the simple organic compounds benzene and toluene. In 1952 Baker prolonged allograft survival by administration of nitrogen mustards. In 1959 Schwartz and Dameshek initiated the modern era of pharmacologic immunosuppression by documenting that the antiproliferative drug 6-mercaptopurine (6-MP), which was developed by Hitchings and Elion as a competitive inhibitor of purine salvage pathways, dampened antibody production and prolonged rabbit skin allograft survival. In order to avert the susceptibility of the unshielded mercapto-group to gut hydrolysis, an imidazole derivative of 6-MP was demonstrated by Calne under Joe Murray's direction, to prolong the survival of canine renal transplants from 7.5 to 23.7 days. How well I recall the thunderous applause when Calne ended his presentation at the 1962 New York Academy of Sciences meeting by showing the azathioprine-treated dog exercising the prerogative conferred by his successful transplant!



Our members Zukoski and Lee in conjunction with Hume not only confirmed the activity of azathioprine, but also documented the benefit of antiinflammatory corticosteroid therapy—first in the canine model and then in humans—thereby extending Krohn's observations on rabbit skin allografts. For the dozen years 1966 to 1978, "conventional" therapy was the double-drug azathioprine-prednisone combination. Its "slippery slope," although not as steep as radiation, not infrequently caused despair over bone marrow aplasia, gastrointestinal visceral perforations, and/or overwhelming fungal infections. Is it any wonder that members of this society who were initiated into clinical transplantation using double-drug therapy now have great ambivalence in prescribing these toxic drugs that created the "slippery slope" that frequently defied our technical successes?

Two attempts to improve the immunosuppressive efficacy of azathioprine-prednisone were unsuccessful: Godfrey and Salaman documented that local graft irradiation introduced by Wolf et al. actually reduced renal allograft survival. Thoracic duct drainage, originally constructed in rats by Woodruff and Anderson, based upon Gowans' description of the critical role of this avenue in lymphocyte recirculation, was applied to man by Franksson and Bloomstrand in Scandinavia, as well as by many of our members: Newton, Richie and colleagues, Tilney et al., Fish, Fitts et al., and Starzl et al. However, thoracic duct drainage showed an absolute requirement for pre-transplant initiation, was frequently difficult to establish and maintain, and had only transient effects.

The second stage in the immunosuppression timeline focused the attack upon T cells. Antilymphocyte sera produced in 1899 by Metchnikoff were reapplied 70 years later in rodent models by Russell and Monaco and by Levey and Medawar. Rapid translation to the clinical arena by Starzl and Marchioro, with subsequent refinements by Najarian and Simmons, led to powerful polyclonal reagents of high immunosuppressive activity. Although the broad degree of T cell inactivation improved the clinical efficacy, the wide spectrum of susceptible, nonspecific host-resistance elements not infrequently exacerbated the dangerous incline of the double-drug slope, although clinical acumen in its application increased the overall graft success rate.

Monoclonal antibody technology offers the possibility of selective reagents not only to dissect, but also to neutralize, cells bearing specific surface markers. Fortunately, recent work portends advances from the relatively nonspecific bludgeon OKT3, an IgG<sub>2a</sub> monoclonal antibody pioneered by our member Cosimi et al. Although useful, it has been associated with frequent, occasionally serious, and even lethal adverse reactions due to cytokine release and to a remarkable propensity for lymphoma development when administered in conjunction with prophylactic equine antilymphocyte globulin, azathioprine, cyclosporine, and prednisone. Indeed, one must question whether there is any real indication for OKT3 use, since it certainly has not shown superior results to the previous polyclonal reagents and since there is not infrequent production of antimouse antibody that will prevent patients from receiving second-generation murine monoclonal antibodies. Four new selective reagents include the IgG<sub>2b</sub> anti-T-cell receptor reagent, produced by Kurrle and used in Europe by Land and colleagues and by Wonigkeit and Pichlmayer and in the U.S. by our



Houston group. Because it may react with a common epitope on the TcR, this monoclonal antibody may interfere with T cell triggering, thereby deviating the antidonor response toward an ineffective, anergic pathway. Anti-CD4 antibodies that have been used in animal models, singly and in combinations, are being prepared for clinical trials. Anti-IL-2 receptor reagents that may selectively destroy activated cells have been administered per se by Cantarovich et al. and by Soulillou et al. in France, and in the U.S. by our member Kirkman, as well as in immunoconjugated form. Chimeric human Fc-mouse F(ab2)' monoclonal antibodies with anti-CD 7 specificity have recently begun clinical trials. Although elimination of the murine Fc piece reduces, it does not abrogate the possibility of patient antibody production toward variable-region determinants, an antiidiotypic response that vitiates the possibility of repeat treatment. In addition, the high incidence of vascular complications (3 of 15 patients) producing graft loss suggests a greater propensity of Fc-receptor bearing elements to mediate reactions toward the human Fc pieces on the chimeric human/mouse anti-CD7 antibody.

The third stage of immunosuppressive therapy utilizes agents that inhibit cells regulating the maturation of alloreactive immune elements. The prototype cyclosporine was isolated in 1969 from *Tolypocladium inflatum Gams*, a member of the *Fungi imperfecti*, contained in soil samples derived from Hardanger Vidda, a high treeless plain in southern Norway. Although it had little promise as an antibiotic, Jean-Francois Borel resurrected cyclosporine as a potent immunosuppressive agent in transplantation and autoimmune disease models. David White et al. documented that short-term administration markedly prolonged allograft survival in animals. On the one hand, cyclosporine inhibits lymphokine synthesis and cytotoxic T cell generation; on the other hand, it spares suppressor T cell maturation. Although cyclosporine displays an amazingly low immunosuppressive hazard and a secure path on the "slippery slope" of therapy, its array of pleiotropic nonimmunologic nephrotoxic side effects prevents administration of sufficient doses to fully exploit its potential in transplantation. However, cyclosporine has provided the major, much-needed impetus for the transplant enterprise. It has kindled the development of new immunosuppressive agents: pharmacologics such as deoxyspergualin, mycophenolic acid analogs, and the lipophilic carboxy-cyclic actinomycete macrolides FK506 rapamycin, and molecular mimics such as cytokine receptor analogs.

Future rational use of immunosuppressive agents will depend upon elucidating their individual molecular targets and pharmacologic interactions. At the level of the surface membrane, the molecular targets are presumed from the putative specificity of each monoclonal antibody. However, their individual mechanism of action may be more complex than simple receptor "blindfolding," endocytosis, or shedding. At the level of membrane transduction to nuclear activation, corticosteroids inhibit m-RNA transcription (such as IL-1 $\beta$ ) via specific DNA steroid response elements. Cyclosporine and FK506 probably inhibit related regulatory DNA-binding proteins, which are necessary for enhanced transcription of T cell activation genes. While peptidyl prolyl cis-trans isomerases bind cyclosporine, FK506, and/or rapamycin, increasing data suggest they are not the exclusive target of drug action. Finally, aza-



thioprine and mycophenolic acids prevent DNA synthesis by inhibiting enzymes of the purine salvage pathways. It is not unreasonable to expect that by the end of this decade, synergistic immunosuppressive drug combinations will produce negative regulation of T cells with minimal toxic side effects, akin to the principles widely applied in cancer chemotherapy. However, this impenetrable shield over the specific immune system almost inevitably engenders risks of neoplastic and infectious diseases.

### Tolerance Timeline

The ultimate immunosuppressive therapy selectively depresses host reactivity toward foreign donor antigens by inducing immunologic tolerance: specific donor, but not third-party, grafts survive without the need for chronic immunosuppression. The tolerance concept originated with the observation that fetal hosts exposed to foreign cells lost their immune responses to donor antigens. In 1914 John Murphy reported the outgrowth of Rous chicken sarcoma cells upon the chorioallantoic membranes of duck or pigeon egg embryos, but not upon implantation into adults. The unresponsive state of the embryo was reversed by inoculation of adult chicken lymphoid cells, particularly small lymphocytes. Demonstrating that the synchorial placenta of freemartin, nonidentical calf twins described by Lillie permitted blood exchange, Ray Owen proposed that mutual tolerance was acquired by fetal exposure to “nonself” constituents. Billingham et al. extended Murphy’s observations in inbred mice, Hasek verified the concept with membrane bridges between chicken egg embryos, and Woodruff confirmed the state in newborn rats.

Burnet then replaced his earlier “self-marker” theory, which suggested that host cells bore a marker that identified them as “self” and thereby protected them against attack by lymphocytes, with a clonal selection theory: The immune system is purged of “self” (auto-) reactive lymphoid clones during ontogeny. The “central” thymic purging process includes two steps: positive selection of T cells recognizing antigen in a context of “self”-MHC products, and negative selection (deletion) of T cells reactive with body constituents. Similarly, autoreactive immature B cells are inactivated in the bone marrow by deletion. Kappler et al. showed that transgenic mice, the bone marrow-derived cells of which synthesize the I-E antigen, which was not normally expressed in those hosts, only displayed specific amino acid sequences in the variable portion of the  $\beta$  chain ( $V\beta$ ) of the TcR on lymphocytes in the thymus—and not on peripheral T cells. This observation elegantly supports the concept of clonal deletion in the thymus. A therapeutic extension of this hypothesis achieved remission of experimental allergic encephalomyelitis by depleting cells bearing TcR with specific  $V\beta$  sequences. Thus, a “natural” mechanism of self-tolerance is inactivation of immature elements upon contact with antigen in a central lymphoid organ. These observations suggest that the continuous presence of donor “non-self” antigen in the central immune compartments of the recipient maintains a balanced state of host-versus-graft and graft-versus-host tolerance.

Chimerism is not a feasible clinical strategy. It demands “debulking” the immune system to provide “space” for the second population, deplete peripheral lymphoid



cells, and recreate the "pristine" fetal state. In animal models, chimerism has been established after total-body or total-lymphoid irradiation (TLI), particularly in combination with donor or Fl bone marrow cells. However, adult allogeneic T cells in the bone marrow pose the hazard of graft-versus-host disease unless "purged" from the donor inoculum with anti-theta 1.2 antisera or by use of nu/nu (T cell-deficient) donors. Hematopoietic chimerism seeds donor-type dendritic cells into the thymus, thereby negatively selecting (removing) donor reactive T cells. Thus, hosts displaying the "chimeric" type of tolerance show either an absence or a changed repertoire of donor-reactive cells, in some instances associated with increased numbers of  $\gamma\delta$  TcR+ elements.

Can tolerance be produced without the bludgeon of "debulking" and/or the reversion to the pristine fetal state? The "horror autotoxicus" concept of Ehrlich postulated tolerance to be a natural process within the immune repertoire, suggesting that lymphocytes could be rendered tolerant even after they had left the thymus. In the 1920s Felton showed that administration of high doses of slowly metabolized, pneumococcal polysaccharide induced unresponsiveness, rather than immunity, upon rechallenge with antigen. A decade later Sulzburger as well as Landsteiner and Chase found per os administration, rather than percutaneous application, of antigen evoked unresponsiveness rather than delayed-type hypersensitivity. Martinez and colleagues tolerized murine hosts toward allogeneic skin grafts with multiple intravenous injections of F<sub>1</sub> cells. This "peripheral" form of tolerance occurs in spite of the presence of T cells that have the potential to recognize alloantigen. Transgenic models elegantly document the phenomenon: fertilized mouse eggs microinjected with constructed restriction enzyme fragments encoding foreign alloantigens are transferred into pseudopregnant Swiss mice to mature into native-type animals bearing unique markers. In spite of foreign I-E class II molecules restricted in expression to the acinar pancreas and kidney, or to pancreatic islet beta cells, these tissues do not elicit rejection responses. T-helper cells react *in vitro* to the alloantigen, but are presumably inactive *in vivo* due to a reduced affinity or altered activation capacity for the foreign antigen. Adoptive transfer of T cells from normal virgin hosts kills the transgenic cells bearing foreign alloantigen.

Three mechanisms may explain post-thymic "peripheral" unresponsiveness: "veto" cell generation, T cell anergy and/or suppressor cell action. "Veto" elements possibly bearing a special form of donor antigen inactivate precursor, but not mature effector, alloreactive cytotoxic T cells, in a fashion resistant to exogenous costimulatory factors. While "veto" activity was not documented in a minor histocompatibility system, Thomas et al. reported that this mechanism mediates allograft survival in hosts conditioned with antilymphocyte serum and donor bone marrow inocula, and Martin and Miller as well as Van Twuyver et al. implicate it in the generation of unresponsiveness after pretransplant allogeneic lymphocyte transfusions.

Anergy represents an unresponsive state of antigen-reactive lymphocytes. Bretscher and Cohen proposed that this unresponsiveness is due to a failure of helper-inducer T cells to produce the appropriate second humoral activation signal. T-helper cells recognize alloantigen, but neither proliferate nor secrete IL-2, addition of which



reverses the anergy. On the one hand, anergy may result from ineffective stimulation, for example following treatment with immunogens modified by crosslinking fixation or by planar membrane array. On the other hand, it can be produced by direct contact of antigen with immature T or B cells without suitable presentation. The critical role of the helper cell second signal to B elements was documented by the failure of B cells expressing the transgene-encoded, membrane immunoglobulin to secrete antibody upon confrontation with the homologous chicken lysozyme epitope. This hyporesponsive state was associated with decreased surface membrane IgM (but continued membrane IgD) expression—a not uncommon phenotype among normal spleen cells, possibly representing anergic B elements.

Some rodent and/or canine tolerance models abrogate helper-inducer function by nonspecific bludgeons: massive doses of single or multiple monoclonal antibodies in combination with cyclosporine, or, alternatively, large cyclosporine doses alone. However, the staggering morbidity of strategies based solely upon nonselective attack upon T-helper elements is unacceptable: such intense immunosuppression clearly provides a setting for infection.

Gershon and Konda documented a third “infectious” tolerance mechanism based upon the capacity of lymphoid “suppressor” cells to adoptively transfer unresponsiveness. One theory proposes that suppression is mediated by a distinct cell lineage—namely, one CD4<sup>+</sup> inducer and two MHC-restricted CD8<sup>+</sup> effectors—as proposed initially by Dorf and Benacerraf and translated to rat alloimmune reactions by Hutchinson et al. Utilizing *in vitro* allostimulation of human lymphocytes, Engelman and colleagues described populations of distinct phenotypes: an “inducer” CD4 Leu 8 population that activates effector CD8 Leu 9.3 suppressor cells—possibly similar to those found in TLI-conditioned, donor-unresponsive, long-term renal allograft recipients. An alternate theory suggests that suppressor activity does not reflect a distinct lineage, but rather a response within the differentiation repertoire of all cells.

Suppressor activity has been implicated in seven tolerance models: animals rendered neonatally tolerant to class II MHC antigens show a higher frequency of tolerogen-reactive lymphocytes than normal mice, yet their cells transfer an unresponsive state to naive mice but mediate neither cytotoxicity nor delayed-type hypersensitivity. TLI treatment induces both donor-specific and nonspecific suppressor components. “Debulking” strategies of tolerance induction using cyclophosphamide to produce clone stripping show initial deletion evolve to suppressor mechanisms. Suppressor cells effect the unresponsiveness produced by ALS combined with bone marrow, as well as by high-dose cyclosporine treatment, particularly in combination with extracted antigen. Finally, suppressor T cells—possibly of the CD4<sup>+</sup> phenotype—mediate classic humoral unresponsiveness associated with “enhancing” antibody.

Suppressor cells may act via unique “processed” donor antigen, production of humoral inhibitors, or antiidiotypic mechanisms eliminating cells bearing donor-specific TcR. Antiidiotypic,  $\alpha/\beta$  TcR<sup>+</sup> CD3<sup>+</sup> CD8<sup>+</sup> cells specifically proliferate upon confrontation with allo- or antigen-specific TcR. Batchelor et al. demonstrated that CD8<sup>+</sup> spleen cells in rats bearing long-term allografts adoptively transferred *in vivo* suppres-



sion, and proliferated in vitro upon confrontation with syngeneic lymphocytes bearing the anti-donor TcR idiotypic.

In vitro assays may differentiate deletion or veto from anergy or suppressor mechanisms in unresponsive individuals. The latter but not the former two phenomena are vanquished by in vitro mitogen activation of, or exogenous cytokine addition to, cytotoxic lymphocyte precursor frequency assays (f[CTLp]). For example, the low anti-donor f(CTLp) in one long-term allograft recipient was reconstituted to a fully expressed  $\alpha/\beta$  and  $\gamma/\delta$  TcR repertoire upon in vitro activation. Also, f(CTLp) assays suggest a contribution of suppressor cells if there are biphasic profiles showing paradoxically reduced cytotoxic responses at high cell numbers. Although the f(CTLp) assay proffers a ready tool to predict and monitor alloreactivity, there are two areas of concern: First, the genetic and environmental variation in the frequency and specificity of reactivity among healthy volunteers and between mouse strains is both considerable and unexplained. Second, two congenic rat strains have been shown to display identical f(CTLp) values, in spite of widely disparate donor allograft survivals in vivo.

The mixed lymphocyte reaction, an in vitro model of allojection, may show proliferation of cells from hosts displaying the anergic form of tolerance—namely neonatally class II-tolerant animals as well as successful renal and bone marrow allograft recipients. Suppressor mechanisms may be documented in vitro using post-transplant recipient cells to dampen in vitro antidonor MLR and/or CML responses by the patient's own pretransplant lymphocytes (the "three cell assay"). Because in vitro activities correlate poorly with in vivo events, systemic transfer experiments remain the gold standard of "suppressor" activity. Thus, the past three decades have witnessed the progression of tolerance investigations from intact hosts to in vivo transgenic and in vitro cellular models. Future dissection of tolerance mechanisms will undoubtedly rely upon incisive molecular technologies.

*Extracted antigens as tolerogens.* One approach to induce tolerance disrupts allorecognition of foreign tissue at the antigen level. Reduction of surface antigen content by somatic point mutation, inhibition of a regulatory protein, or insertion of a repressor homeobox gene sequence protects targets of alloimmune reactions, but has only remote clinical application. Contrariwise, manipulation of the foreign antigenic stimulus to deliver a signal that induces lymphocyte unresponsiveness rather than activation has great clinical potential. This timeline begins with Medawar's observation that pretreatment with semisoluble, crude antigenic extracts modestly prolonged the survival of donor-type murine skin grafts. He suggested that truly soluble extracts might induce tolerance, since they proffer an "unnatural" form of foreign epitope that may "deviate" the host to an ineffective immune response. Dresser had previously shown that administration of soluble monomeric, but not sedimented aggregated, gamma globulin induces antigen-specific suppression of the immune response. Weigle and colleagues found that the rapid and durable development of peripheral T cell tolerance to monomer was due in part to anergy from defective triggering of IL-1 production. Some investigators suspect, but others doubt, the participation of suppressor cells in the phenomenon.

On the one hand, administration of bone marrow or other intact cells, platelets,



subcellular membranes, or transfected cells bearing foreign class II MHC alloantigen induces unresponsiveness. On the other hand, extracted antigens have numerous potential advantages for this purpose, since they are less likely to carry unacceptable, unpredictable risks of sensitization; unable to replicate, therefore posing no risk of graft-versus-host disease; molecularly well-defined, with only a limited array of epitopes; likely to display altered pharmacokinetic or immunologic metabolism possibly bypassing tissue or nodal structures; and susceptible to chemical modification to cover immunogenic and/or reveal cryptic suppressogenic epitopes.

Medawar's prophecy has not been entirely fulfilled: pretreatment with putatively soluble materials prepared by low intensity sonication, by salt extraction, by detergent dispersion, or by papain hydrolysis produced only modest prolongation of allograft survival. Large amounts of detergent-stabilized, class I protein micelles only prolonged rat allograft survival when administered one week before, but not at the time of, transplantation. An unequivocally soluble, cytosolic form of class I antigen extracted from rat, but also present in human liver cells, which lacks the hydrophobic transmembrane domain because of alternative mRNA splicing of exon 5, induced only modest prolongation of survival in some, and had no effect in other trials. Indeed, a 200 ng/ml serum concentration of foreign, soluble, truncated class I antigen endowed by transgenic methods did not by itself achieve allotolerance. These extracted materials bear immunogenic activity: they induce accelerated rejection of donor allografts *in vivo*, and both detergent-dispersed and genetically engineered antigens activate T cells *in vitro*, displaying a high affinity for their TcR ( $kD = 0.1 \text{ mM}$ , 385). Thus in spite of extraction, these materials *per se* preferentially trigger T cell activation.

In order to mitigate the activation pathways, donor extract treatment has been combined with adjunctive immunosuppressive agents: namely, hydrocortisone, polyclonal antilymphocyte sera, cyclophosphamide, cyclosporine, and TLI. One injection of 3M KCl extracted antigen the day prior to transplantation combined with three cycles of three *per os* doses of cyclosporine (10 mg/kg) produced permanent survival of 40% of rat renal (but not cardiac) allografts. Repeat-donor, but not third-party, skin grafts were accepted by these unresponsive hosts. The phenomenon appeared to be mediated by antigen-specific suppressor cells documented by both systemic adoptive transfer assays and *in vitro* tests. At ten days after transplantation, host splenic suppressor elements dampened MLR and CML performances, in spite of a normal *f*(CTLp) upon limiting dilution analysis in the presence of exogenous IL-2. Because this assay did not show a biphasic pattern, there appeared to be an additional component of anergy. Multiple intravenous antigen injections combined with cyclosporine prolonged survival of cardiac allografts. TLI (1600 rads) provided more potent immunosuppression in combination with one injection of 3M KCl extract the day before transplantation. There was uniform, indefinite, donor-specific, cardiac allograft survival. The unresponsive hosts contained donor-specific, suppressor spleen cells that adoptively transferred a state of total and permanent unresponsiveness to syngeneic virgin hosts and produced biphasic *f*(CTLp) patterns.

A second approach to mitigate T cell activation seeks to present extracted antigen



under conditions suboptimal for T cell receptor stimulation or for binding by accessory coreceptors LFA-1 or CD4/CD8. Monovalent peptide fragments rather than multivalent transplantation antigens may cause occupancy, yet produce functional inactivation of T cell receptors. HLA peptides of  $\alpha$ -helical structure, particularly with a central tryptophan, bind T cells. Schneck et al. found that both  $10^{-7}$  M intact soluble class I molecules and  $10^{-4}$  M amino acid 163-174 peptides inhibited a weakly crossreactive H-2 response. Residues 61-69 of a synthetic H-2K<sup>b</sup> peptide arrayed on Ia-bearing antigen-presenting cells selectively activated helper elements. Further,  $10^{-4}$  M of a synthetic peptide mimicking amino acid residues 98113 of the HLA-A2  $\alpha_2$  domain specifically inhibited target cell recognition by CTL, in the fashion of a free hapten. In vitro CTL reactions discriminated among mutant H-2 or HLA peptides differing by only three (152, 155, and 156) amino acid residues in the  $\alpha_1$  or a similar restricted length in the  $\alpha_2$  domain. Furthermore, a naturally processed 10-15 amino acid, H-2 peptide complex extracted from the cytoplasm of antigen-presenting cells bound to T lymphocytes. These experiments suggest that peptides occupying (without crosslinking) T cell receptors potentially produce T cell inactivation. In addition to the possibility of presenting native peptides, host CTL might be subverted by introducing related, but immunologically noncrossreactive, peptides that either deviate the reactivity of existent cells bearing clonotypic receptors or lead to the assembly of competing peptide-histocompatibility antigen complexes.

A third tolerance strategy seeks to enhance suppressogenic or mask immunogenic domains of the major histocompatibility antigens. Antigenic determinants are classified as either linear (continuous) epitopes composed of 2 to 8 (average, 6) residues in the primary amino acid sequence, or discontinuous epitopes conformationally stipulated by molecular folding and side chain association. Although analyses using synthetic peptides bearing individual amino acid substitutions combined with specific monoclonal antibodies suggest the entire protein surface is potentially antigenic, T cell responses toward model antigens, including hen egg lysozyme, sperm whale myoglobin, cytochrome C, and staphylococcal nuclease are in fact directed toward only a restricted number of immunodominant epitopes. The immunodominant epitopes are readily exposed during antigen processing; crossreactive with epitopes seen during previous bacterial infections; amphipathic, firmly anchored structures, and/or avidly bound by MHC molecules in the fashion of "superantigen" complexes. Peptide analysis, as well as work using domain-shuffled molecules, suggests that allorecognition is effected by nonlinear epitopes created by conformational interactions.

Similarly, there is evidence of specialized suppressogenic molecular regions. Sercarz et al. found that amputation of the N-terminal peptide on hen egg lysozyme that by itself induced tolerance via multiple mechanisms, including suppressor cell generation and clonal inactivation, converted "nonresponder" H-2<sup>b</sup> mice to "responder" hosts toward the immunogenic, amino acid 46-61 domain. Suppressogenic epitopes separated by isoelectric focusing from immunogenic determinants within crude 3 M KCL extracts of tumor cells enhance neoplastic outgrowth. Furthermore, insertion of trinitrophenyl epitopes onto the surface of intact rodent cells modestly prolonged subsequent donor graft survival. Zhang et al. reported that peptide fragments of



bovine serum albumin bind murine antigen-specific suppressor, but not helper, T lymphocytes. Presumably molecular probes could target subtle differences in the epitope or conformational specificity of  $\alpha/\beta$  TcR formats on CD4 or CD8 suppressor versus helper/cytotoxic elements. A combination of tools, including chemical dissection overlapping synthetic peptides, x-ray crystallography, and genetic analyses by exon shuffling, may be applied to design strategies to modify the chemistry, size, and polarity of extracted transplantation antigens or their peptides.

A more incisive approach utilizes site-directed mutagenesis to prepare hybrid antigens bearing point amino acid substitutions, as probes of molecular fine structure for testing in transplant models, either via transgenic animals or as extracts of production vectors. These mutations may alter binding of agretopic residues to MHC molecules on the antigen-presenting cell, or epitopic residues to the TcR. Successful combinations of molecular modeling with site-directed mutagenesis by Roberts et al. enhanced antibody affinity, and by Good et al. produced more immunogenic *Plasmodium falciparum* circumsporozoite proteins. In fact, site-directed mutagenesis of a fragment of genomic HLA-B27 DNA at position 67 has already been shown to produce side chain size distortion in the  $\alpha_1$  domain helix, thereby reducing antibody binding. Systematic application of site-directed mutagenesis to uncover suppressogenic versus immunogenic epitopes may elucidate the microstructure of transplantation antigens and afford insights into rapid, direct, chemical methods to treat fresh cadaver donor subcellular extracts in order to obtain tolerogenic materials.

### Prospects for the Coming Decade

Just as the aforementioned confirms the scientific progress toward understanding and manipulating the biochemical basis of individuality, so will rigor in clinical investigations promote our goals: science demands it, our patients deserve it. "Clinical investigation by testimonial" in the present limelight only sabotages the transplant enterprise, at a time when our society is facing unprecedented challenges. My presidential year began in June 1989, addressing a (thankfully) unsuccessful, New York State legislative proposal that raised the specter of an additional level of governmental regulation. In fall 1989, our society commenced an initiative to rectify the inequitable financial compensation for renal transplantation. Not only has this problem existed for almost two decades, but also federal physician payment reform legislation threatens to exacerbate it. Through a consensus-building process on visit patterns, by direct administrative contact, and via written testimony, ASTS delineated many unique aspects of transplant practice. The Physicians Payment Reform Commission and the Health Care Financing Administration have now both recognized the need to develop a specific relative value scale for our procedures in relation to other surgical operations. During the present Congressional session, ASTS endorsed, but made suggestions for amendment of, the 1990 reauthorization bill for the National Transplant Act of 1984. We recommended discrete funding for demonstration projects to test new approaches to organ donation and continuation of the Organ Procurement and Transplant Network (OPTN), as well as extension of Medicare coverage of immuno-



suppressive drugs from one to three years, thereby co-terminating with federal disability benefits. In May 1990, a position paper was developed by a panel of our members in response to the proposed "Medicare Regulations for Liver Transplantation." Although we concur with the procedures and criteria for center selection, we objected to the excessively truncated proposed list of "indications" for liver transplantation. Later this week, we address the educational challenge with the first Postgraduate Course, which will instruct members and their fellows in the Excalibur of our Society—immunosuppressive therapy. It is our skill to wield this sword that distinguishes us from "uninitiated" surgeons. Through this eventful journey our new vehicle *The Chimera* has updated the membership.

These challenges wane compared with the major obstacle to our enterprise—the reduced number of organ donors. The problem is multifaceted: first, circumstances unrelated to transplantation have decreased the number of potential donors—namely, seat belt laws, reduced speed limits, cycle helmet regulations, improved trauma care, nursing shortages, handgun rules, and rigorous efforts against drunken driving. A second problem directly arises from recent events: the medical community's fear of latent AIDS infections in donors displaying "high-risk" profiles, and formal opposition to organ retrieval by members of the "pro-life" movement. Third, anecdotal information suggests increased public discomposure about the procurement system, including the ground rules for retrieval, distribution equity, and ownership of organs. Efforts to promote public attention to improve clinical successes have not been complemented by sufficient attention to reasoned public, executive, and legislative discussion of the policy implications of this technology transfer. A fourth problem has been engendered by at least two unanticipated, negative effects of the National Transplant Act. "Required request" legislation—namely the law designed to ensure that a request be tendered to every potential donor's family—has erroneously invested untrained, ambivalent paraprofessionals with the mantra of our enterprise. Has this ineptitude caused the eroding public enthusiasm evidenced by the finding that the major difference between 1989 and 1986 was an increased number of family refusals to organ donation requests? Indeed, the social forces that resist the legislative "fix" of "required request" will most certainly backlash toward "presumed consent." Another unexpected adverse aspect of the legislation has resulted from the creation of monopolistic organ procurement organizations (OPO), particularly in privatized so-called "non-profit" entities, which have distanced the transplant team from a process long recognized to depend upon interprofessional communication and trust. The unfortunate decline in organ donations during the past five years reinforces the wisdom of our intuitive, previous approach which was based upon the American tradition of independence from, rather than dependence upon, legislation. It is hoped that the rapidly deteriorating organ retrieval situation can be reversed by a systematic, rational public health approach to organ donation, based upon the knowledge and expertise of our society's members, rather than ill-founded speculations of neophyte OPO dilettantes.

Although these mechanistic issues have recently intensified the shortages, the continuing problem is that the American public (and their professional representative, the neurologic surgeon) are only "inclined," but not "committed," to organ dona-



tion. Whereas belief comes relatively easy, and true acceptance a bit harder, commitment is much rarer; and the decision to act is the most difficult of all. On the one hand, the unique social circumstances of donor death, wherein 78% of candidates are less than 45 years of age and almost all have been ill for less than 72 hours, emphasize the fragility of life and capriciousness of disease to a society that stigmatizes the ill and disadvantaged. On the other hand, both the public and health care professionals are ambivalent about the brain-death concept. Part of the problem may be semantic. Gaylin's term "neomort" conveys the sense of neonate (newly born) and mort (dead). Confusion is evident when recipients are told that organs are being kept "alive" in a donor who is "dead." The power of language is underscored by public repugnance toward an albeit fictional "bioemporium," the "Jefferson Institute" of *Coma*, a holding ward of neomorts to serve training, experimentation, and transplant needs. Transplantation may thus erroneously evoke the technologic arrogance of Dr. Frankenstein.

Although families rationalize refusal of organ donation requests based upon religious precepts, superstitions, and perceived racial or economic exploitation, I submit that fundamental cultural taboos are more likely sources of resistance to our enterprise—namely, fears about premature termination of life; subliminal coercion; infliction of additional suffering; violation of the sanctity of the body by assault, disrespect, or diabolical pollution; negation of the possibility of resurrection at the Second Coming of Jesus Christ; destruction of the soul/mind/body composite; and the corpse *per se* (and particularly its return). Our culture's traditions demand that due respect be paid to the corpse by the living, in order to ensure the speedy release and future well-being of the departed spirit, particularly during the fraught period after death and before burial.

Can payment for organs in the fashion that commercialization of blood, sperm, and even the rental ("womb space") of body parts—do anything but increase public resistance? While commercialization of organs of living unrelated persons, as practiced in several Asian countries, is generally accepted to be reprehensible, several Mephistophelian alternatives of "rewarded giving" have been recommended for cadaver donor families: direct remuneration, defraying burial expenses, providing insurance, or forgiving legacy duty. The sordid saga of anatomic donation provides a lesson. A perceived scarcity of supply, due to dissection being recognized as a punishment worse than death, was addressed by legions of body snatchers (or "resurrectionists" as they became euphemistically known), including executioners, undertakers, grave-diggers, aspiring surgical students, and eventually murderers ("burkers"), who bartered corpses for any of the aforementioned motives. The public's brittle tolerance of dissection, due to the very traditions regarding the dead and fears of their mutilation cited above, was unfortunately ridiculed by members of the medical profession as "vulgar prejudice," rather than addressed as a legitimate public ambivalence. In the same way, negative aspects muddle the positive "giving" side of organ donation, as emphasized by Youngner.

O'Flaherty distinguishes two pancultural motifs: the hunter, a person who has to experience everything physically, and the sage, one who uses mental powers to learn about other people's lives. The distinction is reminiscent of my father's adage: "He



who learns from his own experience is a wise man; he who learns from others' experiences is even wiser." The sages whisper that entrepreneurial medicine that regards organs as a commodity has no role in our enterprise. Commercialization or "rewarding" demeans the spiritual value of the act. Indeed, it creates exactly the undesired impression that the body is a token of exchange subject to commercial dealing, rather than an object worthy of respect.

The sage might reason that the failure to declare a positive act of commitment results from the vacuum in the ethical and moral fabric of what Joseph Campbell calls the demythologized American society. Myths no longer shape our lives with meaning and concern; rather, outer appearances may go so far as to overwhelm inner spiritual values. Is the lack of a shared, meaningful American mythology or imagery for the sense of "community" (as opposed to "individual") an inherent societal barrier to organ donation? Is donorship not personally meaningful to families because populist thought views death from the perspective of an individual rather than of a humanity which is joined in nature as well as in culture? Altruistic donorship ratifies the bond between the individual and the human race; it confirms that one has been initiated into the purpose and meaning of life. It recognizes adverse events as being in accord with nature, as representing a challenge to unleash one's spiritual potential. The donation act in the setting of death affirms a life lived within the harmony of society; it recognizes donation as a procedure in accord with the way of nature and not impulsive. In our society, donorship should symbolize the timeless, pancultural theme of rebirth, which was identified by Mircea Eliade as the salve that soothes the spirit to confront, bear, and interpret grief. Donation is a heroic act. It is beyond a human act. It is the extraordinary, albeit final, act of which an ordinary person is capable. The donor (and the family) give life to something bigger than themselves.

Since our culture has denigrated books to a degree only exceeded in Bradbury's *Fahrenheit*, oral tradition must be established via the visual media. The effort is not merely a device to satisfy a medical exigency or to proselytize a political agenda (as some of our legislators have demeaned the problem), but rather an enterprise to weave a new skein in our cultured fabric. The organ donation skein recognizes adaptation to death, a common and inevitable event, as a rite of passage through life. It provides a trusted anchor to face this dark encounter and understand this universal reality. It offers a road map to deal with the mythic situation of brain death by doing something in the best interests not only of the afflicted but also of humanity. It offers a basis for commitment.

Mythologic terms immediately capture the positive value in what appear to be negative events, providing meaning to what would otherwise be a senseless tragedy. The classical heroic myth that from a "given" life comes new life cannot be more literally interpreted than by transplantation. The ritual of organ retrieval is a mythic act, reminiscent of the legendary phoenix that, at the end of its lifetime, is consumed by flames on its newly constructed pyre, only to emerge as a seed, then finally the fully developed bird of the sun. The transplantation enterprise that begins with mythical stories of Ganesha, Pien Ch'iao, Ezekiel, and Cosmas and Damian will thus achieve its



goals through public recognition of the symbolic heroism of the organ donor, whose altruistic act is the ultimate expression of the donor's humanity.

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