PRESIDENTIAL ADDRESS

In Search of the Holy Grail

(Actively Acquired Immunologic Tolerance)

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Certainly a highlight of my career has been to serve as President of the Southern Surgical Association.

As a surgical resident in Buffalo, New York, my professor was John D. Stewart, M.D. (a great surgeon and an even greater

SLIDE 1

teacher). Dr. Stewart was on the editorial board of the *Annals of Surgery*. Thus my first subscription to a surgical journal was to the *Annals of Surgery*. There, in the May and June issues I discovered papers presented at the annual meetings of The Southern Surgical. Each year I gained a number of heroes and teachers from the papers in those issues.

I remember a paper by Sparkman regarding planned cholecystostomy and his careful recording of the history of the subject in a footnote. I read with astonishment the experience of DeBakey and colleagues with renal artery stenosis. They reported 32 cases, but an addendum to the article indicated that, since the paper had been written, another 18 patients had been treated. (Fifty cases of renal artery stenosis!) As a medical student I knew Ed Krementz, but learned of his scholarship from his paper on soft tissue sarcoma. I learned about thyroid disease from Tim Thomas, colon tumors from Isidore Cohn, Jr., carcinoma of the esophagus from Ed Parker, and endocrine surgery from Robert Miles and James Hardy. I reveled in the wide-ranging discussions. I recognized that the Southern Surgical Association was one of the more respected of the learned surgical societies, and that the work presented at its meetings was comparable with that presented

anywhere. It was a pleasure to read these works from the great Southern institutions, which seemed to have music in their names.

This discovery was very important to me. At the time (that is, between 1958 and 1965), I had serious conflicts with my heritage. As a seventh-generation Mississippian residing in the Northeast, I found myself an apologist for the South during the great events of the civil rights movement. It became rather difficult to rationalize the Emmett Till lynching, the Philadelphia murders of the Freedom Riders, the Selma march, and the German shepherd police dogs of Bull Connor. At least every month, if not every week, some new atrocity was either blazed in the headlines or shown repeatedly on television. I felt shame and horror for all my brother southerners, both black and white. I could not fathom this state of terrorism. These did not seem to be the people I knew. There were years in the North during that time when a southern accent was thought to be synonymous with ignorance and racism.

Many times during those years I thought of Quentin Compson. Many of you will recognize Quentin as the brother of Caddy, Benjy, and Jason--the Compson children in Faulkner's *The Sound and the Fury*. Quentin killed himself while a student at Harvard because he could not abide his family's shame. I was never in such danger, but I was dangerously close to renouncing my history. For several years I planned never to return to the South. It was, in part, reading these papers and learning from and about those scholars that led me back to my homeland.

I suppose The Southern is special to all of us in different

ways. My life would not have been the same without the impact of The Southern, which I felt long before becoming a member. Thus this address is a special honor for me, and I wanted to use this occasion to say something profound or prophetic. Unfortunately, I seem to have little to say that is either profound or prophetic.

I remember, as a research fellow attending national meetings, glibly agreeing with peers that the presidential addresses were so terribly boring. We assured ourselves that, should we ever have such an opportunity, we would certainly have plenty to say. So now the opportunity is here, and it seems I have very little to say. Perhaps that is part of being southern.

To me, being southern is a state of mind. It is not determined geographically, nor genetically. Although most southerners reside in the South, many do not. In fact, many have never lived in the South, but they are nonetheless southern. The Vietnam War, for example, made many southerners. American Indians commonly are good southerners. Woodward in his classic book *The Burden of Southern History* suggested that southerners are different because they are the only U.S. citizens who have experienced such terrible defeat, destruction, and poverty. That experience provided an insight that others seldom have.

Southerners know that it is very difficult to discover what is truth, or what is useful, or what is good. They know that you can believe in something with all your heart, that you can live your life according to a set of high moral standards--perhaps with an almost sacrificial code of behavior--and still be dead wrong. They

know that you can devote your life and fortune to a cause and lose (and they frequently do). They know that no matter how firmly you believe something nor how much evidence you have collected to support your position, you may be wrong. They know these things from experience. No one else in this country knows this so clearly. Others commonly seem to believe that they can fight and never lose--that if their intentions are good, the consequence of their behavior will be good.

We know that this is not true. Southerners are perhaps more worldly, cynical, and suspicious, yet somehow more romantic, than other Americans. Many of us tend to be Jeremiahs; more of us are mavericks, less likely to rush to change. Perhaps because we are ambivalent over large or important issues, perhaps because we have no confidence in grand schemes and are suspicious of well-intended plans we tend to spend more time thinking on smaller matters of lesser consequence. We commonly become focused on simple things like civility, honor, devotion, courage, friendship, and integrity, which undoubtedly seem to many to be a waste of time. So, in keeping with this condition and having no grand theme for this address, I shall talk for a while about the subject of acquired immunologic tolerance--a topic which has been of interest to me for some years.

Since the beginning of experiments involving the science that became known as immunology, it was apparent that a being generally did not produce immune reactions against itself. This principle was first clearly stated by the famous theoretician Paul Ehrlich,

who called it *horror autotoxicus* and promulgated the idea that the body had a horror of producing antitoxins reactive with its own tissues. The mirror image of this principle was that the body inevitably recognized foreign macromolecules that gained entrance and then took steps to destroy, remove, or delete them. This process was carried out to restore the internal mileau to normal (i.e., all self). Thus it became clear early in the study of immunology that the immune system had an exquisitely sensitive mechanism for distinguishing self from non-self. It would accept self and destroy non-self.

From an evolutionary viewpoint, this exquisitely sensitive process served humankind well throughout its millions of years of existence, when in almost every instance, foreign material within the organism was bad. It is not surprising that the concept of transplanting tissue and organs was met by severe and unremitting efforts on the part of the host to destroy the transplant. In most instances, even today, efforts at rejection continue for many years, and in the end the immune process succeeds in destroying the graft.

The need for skin transplants for patients with severe burns stimulated experimentation, the results of which ultimately established that skin allografts were rejected inevitably and specifically. Findings from more detailed studies by Medawar established that the biologic barrier to successful transplantation of tissues and organs was primarily an immunologic one. This was a critical insight (more important than it seems today). The

barrier might just as well have been biochemical or nutritional, or some other system failure much more difficult to overcome.

Just as burns of patients stimulated interest in skin transplantation, crush injuries resulting in acute renal failure stimulated interest in artificial kidneys and kidney transplantation. Medawar's critical experiments with skin transplants were performed between 1943 and 1945. In 1947, a temporary kidney transplantation was attempted to allow a young woman dying of acute renal failure to survive long enough to recover. This effort was successful. The kidney functioned for a few days and was removed. The experiment was carried out at the Peter Bent Brigham Hospital by three young surgeons in an examining room with the light of only a gooseneck lamp. Two of these young surgeons,

SLIDE 2

Charles A. Hufnagel and

SLIDE 3

David M. Hume, later became fellows of The Southern.

Thus by 1947 it was apparent that transplants between all individuals (i.e., allografts), excluding those between identical twins (i.e., isografts), would function properly but would inevitably be rejected unless something could be done to modify the recipient's immunologic behavior. One question that arose was whether a person's immune system could be modified in such a way as to accept a specific allograft as self and thus not attack it.

In 1945, Owen recognized that fraternal bovine twins commonly

possess erythrocytes of two types: one representing the autogenous genotype and the other representing the genotype of its twin. This was due to the fact that the placentae of these twins commonly shared vascular anastomoses, and blood was cross circulated in utero. Skin grafts between these "chimeric" twins survived indefinitely. Eventually, a few fraternal human twins were found to be both chimeric and tolerant of skin allografts from each other.

During the late forties, Burnett and Fenner were working on a theory of antibody formation which ultimately came to be termed the clonal selection theory. They were wrestling with the problem of how an individual distinguished self from non-self. In Burnett's scheme, the ability of each individual to become immune to any macromolecule resided in a group of cells he termed clones. Each clone had the genetic capacity to react with one epitope or a group of epitopes. On the basis of Owen's observations, Burnett and Fenner postulated that those clones exposed to self-immunogens during gestation died or were suppressed. This led to clonal "deletion" and tolerance to self.

This theory was easily tested and Billingham, Brent, and Medawar promptly did so. In a study with CBA and strain A mice, they injected six CBA fetuses in utero with tissues obtained from strain A mice. **Five of the fetuses were born, and skin grafts from** strain A mice were accepted permanently in 2 of 5 and for about 75 days in 1 of 5 of these animals. Billingham and colleagues called this phenomenon actively acquired immunologic tolerance. This

simple experiment was quickly confirmed, and the possibility of producing a condition in which tissue from one individual would be tolerated by a genetically different individual was established.

Some 41 years have passed, and thousands of publications have described actively acquired immunologic tolerance and its various aspects. Still, the production of acquired immunologic tolerance in the human being remains the Holy Grail of the transplant surgeon.

The following 12 principles have been established regarding acquired immunologic tolerance (adapted in part from Reference 17):

SLIDE 4

1. The definition has been articulated somewhat more **sharply:** Acquired immunologic tolerance is the specific depression of an immune response to an antigen (or antigens) induced by prior exposure to the same antigen (or antigens). (Note that the immune system is depressed not necessarily abolished.)

SLIDE 5

2. Tolerance can be complete or partial. T cells can be tolerant to an antigen, without B cells being tolerant to the same antigen, and vice versa.
3. Tolerance is said to be split if it exists to one epitope on an antigen but not other epitopes on the same antigen, or to one antigen on a cell but not to other antigens on the same cell.

SLIDE 6

4. Tolerance becomes easier to produce as the genetic disparity between two individuals is reduced.
5. High doses of antigen are more likely to produce tolerance than are low doses, although there is a low-dose ("low zone") tolerance to some antigens characterized by tolerance of helper T cells, but not B cells (i.e., T-cell tolerance).

SLIDE 7

6. The intravenous or intraportal injection of antigens is more likely to result in tolerance than is the intradermal, subcutaneous, or intramuscular route.
7. Soluble antigens are more likely to produce tolerance than are insoluble antigens.

SLIDE 8

8. Reducing the size of an antigen without changing its reactive sites increases its tolerogenicity.
9. Increasing the density of an epitope increases its tolerogenicity.
10. The more immature the host, the easier it is to induce tolerance in that host.

SLIDE 9

11. Tolerance, once established, will persist only as long as the tolerogen persists in the host.
12. Conditioning the host with some immunosuppressive agent may increase the chance of tolerance induction on presentation of an antigen (or antigens).

These principles do not seem to be an exceptional harvest for 41 years of effort. In the past few years, considerable detail has been added by findings in experiments with transgenic animals. The experimental details tend to emphasize the variable nature, complexity, and adaptive capacity of the immune system in spite of its incredible specificity, sensitivity, and reliability. Perhaps an overriding concept in (or corollary of) these recent transgenetic experiments is that if an antigen (or antigens) of whatever origin can be placed inside the organism and kept there long enough, tolerance will ensue.

Of the 12 principles listed, 9 are conditions or recipes designed to make tolerance induction more likely. The first 3 are definitions. These definitions are admirably suited to the usual laboratory models of tolerance, in which some manipulation involving the injection of what should be an immunogen produces a state of reduced responsiveness or nonresponsiveness on secondary challenge by the same immunogen.

These definitions may not be quite inclusive enough for the clinical situation. If a patient receives an allogeneic transplant --implanted with the aid of immunosuppressive drugs or some other conditioning modality--and the graft survives with normal function for many years (perhaps 10, maybe 15 years) with greatly reduced doses of conditioning agents, is that tolerance? If all conditioning agents are deleted and the graft continues to function, is that tolerance? In the former situation probably not, because withdrawal of all immunosuppressive drugs leads to

rejection episodes in a high proportion of such patients. In those individuals in which rejection does not ensue, however, tolerance does exist. Proof of complete tolerance would require further allogeneic stimulation from the original organ donor from which no response was elicited. This experiment is usually impossible because of the lack of appropriate antigen from the donor (and is possibly dangerous as well). I am not familiar with any experiments that address this issue adequately in the human being. Murray and associates addressed this question with dogs bearing kidney allografts of long duration that continued to survive without immunosuppression. They found that when the second kidney from the original donor was transplanted into such a recipient, the first kidney remained in good health while the second kidney was rejected. If the accepted first allograft was transplanted back to its original host, however, it was also accepted. Thus a situation of graft and host adaptation had occurred without true complete tolerance. This condition is probably best defined at least operationally as split tolerance because the host is clearly tolerant to some antigens from the donor, but not all antigens present in either the donor or the specific organ.

The situation of a patient tolerating a healthy, well-functioning allograft without immunosuppression is a highly desirable and completely acceptable clinical outcome. It does occur with some frequency as a result of conditions as yet undefined. I assume that this situation represents tolerance, although it probably is split tolerance.

Natural tolerance does occur in some human fraternal twins. Woodruff and Lennox, following the lead of Owen, found one set of such twins. Each had two blood types acquired presumably from shared placentae (as in cattle). This pair of human twins was tolerant to exchanged skin grafts.

Only two years after the classic paper of Billingham and colleagues, Main and Prehn demonstrated tolerance in mice. They showed that total body irradiation followed by bone marrow transplantation produced tolerance to skin allografts from the bone marrow donor applied 24 to 30 days later. Acquired tolerance in the adult human being was proved recently in a remarkable experiment. Two individuals treated previously for leukemia (one recorded as myelocytic and one as non-lymphocytic) with bone marrow transplant later developed renal failure (7 years and 2 years later, respectively). Both received a kidney transplant from their respective HLA-identical siblings who provided the original bone marrow. Both kidneys functioned normally (1 year and 2 years later, respectively) without the aid of immunosuppression other than 5 mg and 7.5 mg of prednisone per day. There was no evidence of rejection. Thus an ideal way to produce tolerance is by performing a bone marrow transplant before transplanting the needed organ. Unfortunately, the risks associated with marrow transplant remain too great for such use. Thus the necessity to induce tolerance in other ways.

All attempts to produce tolerance in the human being have involved some effort to reduce or irradicate mature or

differentiated lymphocytes, leaving only immature cells. It is hoped, that as these immature cells replace destroyed mature cells, in the presence of an allograft they will become tolerant. Although all these efforts have shown some benefit with some overall improved allograft survival, none have given consistent or predictable results as yet.

In an effort to enhance graft survival in kidney transplant recipients, Hamburger, et al used total body irradiation followed almost immediately by kidney transplant. This was the first of a long series of attempts to destroy mature cells in the hope of inducing tolerance in the cells' replacements by exposure to an allograft. Although this approach produced the desired results in some patients, the total body irradiation was associated with a high mortality rate. This was discontinued when effective immunosuppression with azathioprine became available. Similar results were obtained by others.

A modification of this approach (and a much safer one) was attempted almost two decades later with irradiation of the principal lymphoid-bearing areas, rather than the total body. Supplementation with immunosuppressive agents was also included. Again, some patients were rendered tolerant, but most were not. The responses could not be predicted, and the irradiation treatment was time consuming and expensive. Overall, 1-year graft survival was less than that observed with cyclosporin therapy alone.

A different approach with a similar aim is to deplete the body of mature lymphocytes. The technique involves cannulating the

thoracic duct and removing the circulating lymphocytes by various means. This tack was first studied by Tilney and Murray, but was

SLIDE 10

pursued intensively by Fish and associates over a 12-year period. Again, results showed that some people became tolerant, but most did not. Long-term survival was considerably better than that obtained with standard immunosuppression in these pre-cyclosporin experiments. These studies were also abandoned because of the cumbersome, expensive, and unpredictable nature of thoracic duct drainage, along with the availability of improved immunosuppressive agents.

Another body of work involves the use of an immunosuppressive agent, primarily antilymphocyte globulin (ALG) as an inducing agent followed by transplantation of bone marrow and then the desired allograft, or alternatively transplantation of the desired organ may precede the bone marrow transplant. Results are not unlike those previously described. There is so much literature in this area that an adequate review would be beyond the scope of this presentation. I have personally learned more on this subject from Anthony Monaco and his many colleagues who have studied this process in murine species for almost three decades. Thomas and co-

SLIDE 11

workers have produced tolerance by using this method in primates, and Diethelm and associates have studied the problem in human beings.

SLIDE 12

What has been learned through these extensive and at times heroic studies? It seems clear that complete or split tolerance can be produced in the adult human being. The most elegant such example was production of chimerism by bone marrow transplantation (in treatment of leukemia) followed some years later by successful kidney transplantation without substantive immunosuppression. The percentage of bone marrow of donor origin present was not reported, but presumably it was near one hundred percent. All other methods have used some modifier in the hope of inducing a situation in which the graft (or the graft plus other material) will become a tolerizer. Evidently this does work, but the outcome is unpredictable, unreliable, and not always permanent.

The induction of acquired tolerance by long-term residence of a parenchymal organ allograft associated with immunosuppressive drugs certainly occurs and probably with considerably more frequency than was originally appreciated. Almost all senior transplant surgeons have patients with perfectly functioning allografts of parenchymal organs after 10 to 20 years who receive only homeopathic doses of drugs (and some who received no drugs).

SLIDE 13

I have a patient with perfect renal function now in her 25th year after receiving a renal allograft from an HLA-identical sibling (sister). About 18 years after the transplant she decided to discontinue all drug therapy, which she did without consulting me. She informed me of this some three years later, and now after seven years without immunosuppressive therapy she remains entirely well.

In my own clinic there are several other patients with normal kidney function 15 years after transplantation. They receive very small doses of drugs that probably could be discontinued if there were a safe way to predict outcome. Substantial study has been directed by the Pittsburgh group toward similar patients in recent years for different reasons.

How can the problem of producing acquired tolerance be approached in a predictable and reliable way? Most students of clinical transplantation do not believe that reliable, predictable, cost-effective, or durable organ transplantation will become a reality until this problem is solved. Two new insights concerning the induction of tolerance have come to light recently. These are essentially efforts at understanding some observed natural phenomena and how they may or may not affect tolerance.

One is the phenomenon of microchimerism; that is, a chimeric state but one in which only a rare cell outside the transplanted organ is of donor origin. A certain amount of cell exchange takes place between graft and host, and in patients with longstanding and well-functioning grafts the cells of donor origin can be found in several tissues. This phenomenon is established. Starzl and his many associates have proposed that this microchimerism--or "mixed" chimerism--is responsible for the development of acquired tolerance. A number of studies have been conducted in an attempt to relate microchimerism to tolerance, that is, the lack of need to take immunosuppressive drugs. This has not proved to be a simple process. The presence of microchimerism is not synonymous with

tolerance because a number of patients with this identified state reject the donor organ when drugs are withdrawn. Whether there is a quantitative relationship is as yet unknown. Is a certain percentage of chimerism required before tolerance is complete? How does the presence of chimeric cells produce tolerance?

The second area of study attempts to understand tolerance better by considering a soluble fraction of HLA that is probably involved in the maintenance of self-tolerance. This story begins with Calne et al, in 1969 when they noted that some liver transplants between pigs survived permanently without the aid of immunosuppression. Van Rood, and associates, hypothesized that the liver must secrete some tolerizing substance and that substance was probably a soluble form of HLA. The fact that soluble HLA does exist was proved by neutralizing anti-HLA-A2 sera with sera obtained from HLA-A2 positive individuals. The subject was not further clarified until microsurgical techniques evolved that allowed the reliable transplantation of liver in rats. This made it possible to investigate liver transplants in detail in inbred rat strains. Such studies were performed by Kamada in Calne's laboratory. The results were complex. When livers were transplanted between many combinations of histoincompatible rat strains, some combinations resulted in permanent graft survival, and some with extended but not permanent survival time, while others rejected promptly. Subsequently, graft survival was related to the appearance in recipient serum of some substance which neutralized antisera specific for donor histocompatibility

phenotype. Furthermore, if an animal bore an accepted liver transplant, it became tolerant to skin, heart, and kidney transplants from the same donor which it ordinarily would reject in usual time. Thus, at least some livers are tolerogenic and the tolerogenicity is probably conferred by the secretion of soluble histocompatibility antigens. Such grafts induce tolerance to other organs also, but only to those obtained from the same donor strain. Other experiments have now established that the human liver does secrete soluble HLA, class I antigens, and several investigators have suggested that a successful human liver transplant protects a simultaneous kidney transplant.

The implications associated with these studies led us to believe it would be useful to investigate this system in more detail in human beings. We established an enzyme-linked immunosorbent assay to detect soluble class I and class II HLA. We discovered that pure soluble HLA can be found in and readily isolated from spent dialysate. Subsequent experiments established: (1) Everyone has a relatively stable soluble fraction of HLA-I and HLA II in their body fluids, but some have high and others low concentrations, (2) Soluble HLA-I levels rise with rejection and infection, and this soluble HLA-I has some reasonably predictable characteristics in the posttransplant period.

The studies have now been extended to measure some limited number of soluble HLA-I allotypes. Results of these experiments confirm that some of the soluble HLA-I in the serum of a liver recipient is of donor origin and remains of donor origin for some

time. It is not known whether this may prove to be permanent, nor is it known whether soluble HLA of donor phenotype induced by liver transplantation actually comes from the liver for protracted periods. Its production certainly could be translocated to other areas as postulated by the microchimerism theory. Soluble HLA of donor origin also appears transiently in the circulation of renal and cardiac allograft recipients, at least during times of allograft injury, and possibly at other times as well.

These considerations lead to the following hypotheses:

SLIDE 14

The maintenance of self-tolerance is in part preserved by the constant bathing of all tissue in soluble HLA.

SLIDE 15

There is some means of maintaining fairly stable levels of soluble HLA in each individual, but some persons carry higher levels than others.

SLIDE 16

The quantity of each allotype within the total of soluble HLA varies between individuals, and this is in part determined genetically.

SLIDE 17

A means of maintaining the proper concentration of soluble HLA in circulation would lead to tolerance to the allotype and phenotype of the soluble HLA.

SLIDE 18

It is possible that allogeneic cells tolerated by a host

(chimera) maintain tolerance by the production of allogeneic soluble HLA. In turn, the degree of chimerism and the concentration of soluble allogeneic HLA should be related.

These hypotheses do not contradict any known fact. They are compatible with current theories of mixed chimerism or microchimerism. They are subject to scientific testing, and I hope they will be tested soon.

When the Holy Grail is found, human transplantation will become safe, predictable, durable, and much less expensive. It will certainly be worth the odyssey no matter how long. It has been a great privilege for me to be a soldier in this army of scholars for some 32 of the 41 years of study.

Again, I thank you for the honor of being a member of the Southern Surgical Association and serving as your President.