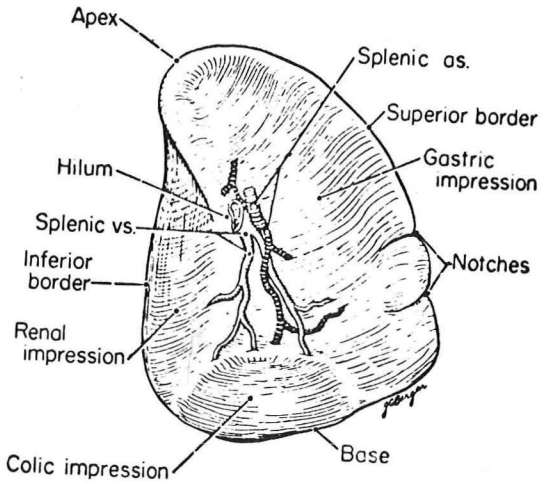
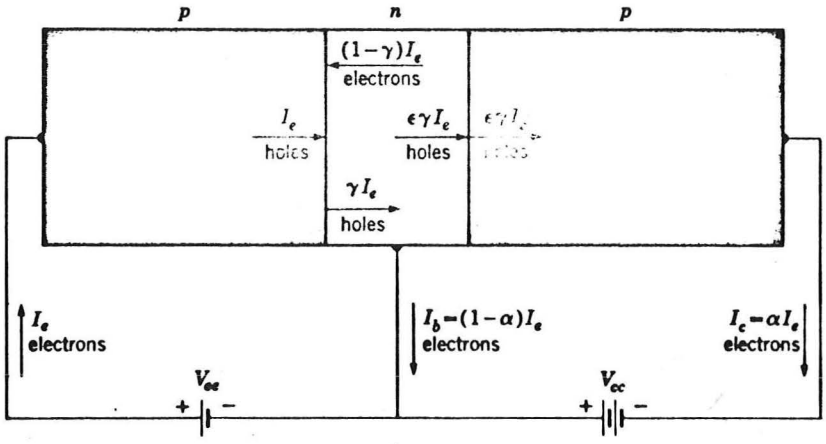
THE SPLEEN:



CLINICAL IMPLICATIONS OF ITS ROLE AS A BIOLOGIC TRANSISTOR



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J. Wayne Streilein, M.D.

THE SPLEEN: CLINICAL IMPLICATIONS OF ITS ROLE AS A BIOLOGIC TRANSISTOR

Some time ago - before the advent of L-DOPA, but decidedly after the discovery of Penicillin - a raw, green, naive medical student heard an erudite member of the faculty of the University of Pennsylvania School of Medicine relate that there remained only three major organs in the human body to which no function could reasonably or reliably be ascribed: the pineal, the thymus and the spleen. As the years have intervened since that poignant moment, the thymus has given up its magic secret: it has captured the imagination of immunologists and biologists the world over by presiding over the one pivotal and crucial event in immunologic development: the recognition of self from non-self. Within the more recent past, the pineal has come into its own as a major determinant in the regulation of neuroendocrinologic transmission, a more realistic though perhaps more pedestrian vision of this uniquely located gland than Rene Descartes had hoped for. And the spleen remains.

The spleen remains, as Galen suggested, the organum plenum mysterii. Precisely why he singled out the spleen for this title when he knew or understood substantially less about the other organs remains unclear. Suffice it to say, that for generations the spleen has been regarded as an organ that "vented" itself. What the initiator(s) of this venting process was, or of what indeed venting consisted has remained totally obscure. With the coming of post-Oslerian medical practice, the spleen has assumed a singular and significant clinical role if for no other reason than that very often in disease it can be palpated. In large measure, knowledge of the functional capacities of the spleen have taken an inferior position to the ability to demonstrate it on abdominal examination. Clinical prowess and acumen are often measured by an individual's ability to identify a 1 finger breadth spleen. There are even some who believe that this peculiar organ in the left upper quadrant serves only to remind medical students of their lowly estate vis-a-vis a more senior physician who invariably finds a spleen that "isn't there" while failing to verify one that "is".

Over the past 100 years, various special interest groups have claimed the spleen for their own, but only three major groups now hold significant claim: the hematologists, the immunologists, and those interested in infectious disease. The hematologists have claimed that the spleen makes new blood cells and destroys old ones, that it stimulates the bone marrow to renewed and vigorous hematopoiesis, while at the same time maintaining a suppressive effect on marrow hematopoiesis. While each of these claims has inherent merit, one is struck by the ludicrous paradox of an organ with such mutually exclusive functions. The immunologists have perhaps staked a more impressive claim to splenic function, but since this brazen new discipline has also laid claim to such diverse organs as the skin, the brain and the appendix, its entire repetoire of putative immunologic organs is somewhat suspect. The very best evidence that the spleen is essential for the maintenance of health comes from the work of those interested in tropical infestations with parasitic organisms, but since their works are published primarily in the Journal of Tropical Diseases (as opposed to the Journal of Clinical Investigation) it seems unlikely that the object of their study could be of prime biologic significance.

As a practicing immunologist, and a nascent-if-blundering hematologist, I must warn you that I am not about to advance a thesis that the spleen plays some heretofore unsuspected role in gastroenterology or neurology! Rather, I would like to examine briefly the current state of our knowledge about the spleen and its functions, to gather together some disparate observations about the spleen which, when examined by Occam's rasor, lead to a rather stark and straightforward hypothesis about the role of this organ. As a test of this hypothesis, I will then

consider certain clinical predictions which should follow if the thesis is correct.

ANATOMY OF THE SPLEEN: GROSS & MICROSCOPIC CONSIDERATIONS

The adult human spleen is a 125-200 gm organ, shaped roughly like the slightly cupped hand without fingers, the indented face containing the hilar structures: splenic artery, vein, and lymphatic vessel (1). On cut section, the spleen can be seen to consist predominately of red-purple tissue (red pulp) throughout which are strewn smaller masses and nodules of whitish appearance (white pulp). Under the microscope, the white pulp consists essentially of densely packed lymphoid tissue through which small arteries can be seen to pass. The presence of lymph follicles with germinal centers indicates that the organization of this lymphoid tissue is very similar to that found conventionally in peripheral lymph nodes. At the periphery of the white pulp the marginal zone allows for the transition to red pulp which can be seen to consist of all the formed elements generally found in the peripheral blood as well as mast cells, macrophages, and plasma cells. The red pulp is also laced with venous sinuses lined by reticuloendothelial cells which help to define the essentially formless melange of cells that lie outside the venous sinuses, but within the red pulp, and have been given the epinymic designation, Billroth's strands (2).

While controversy has raged for years concerning the blood circulation to and through the spleen, this much now seems established (3): the large volume of blood delivered to the organ by the splenic artery is first altered by its course through the central arteries of the white pulp. Here, the small arterioles that branch almost at right angles effectively skim the plasma and white cells from the blood as it flows in typical laminar fashion. As a consequence, lymphocyte-rich plasma is delivered directly into the extravascular lymphoid tissue of the white pulp. The progressively-more-concentrated erythrocyte suspension that coarses through the arterial vessels entering the marginal zone has one of two immediate destinies: a small proportion of the thickened and viscous blood flows directly into the venous sinuses, while the bulk is deposited extravascularly into the marginal zone and Billroth's strands. In order to regain the general circulation via the venous sinuses, the extravascularly placed blood cells must percolate slowly and tediously through the maze of the Billroth strands and ultimately insinuate themselves between the lining reticuloendothelial cells of the venous sinuses. The contents of these sinuses finally leave the spleen through the splenic vein. A primitive network of lymphatics culminates in a lymphatic vessel that exits from the hilum and carries but a modest contribution of lymphocytes to the general circulation via the thoracic duct. The predominate mode of exit for any cell - erythrocyte, lymphocyte, granulocyte, or platelet - is through the splenic vein. Smooth muscle fibers, through their collagenous attachments to the splenic capsule of peritoneum, respond to epinephrine by contracting and "squeezing" a portion of the splenic contents into the circulation, a capability more developed in some species than in others.

FUNCTIONS OF THE SPLEEN

HEMATOLOGIC ASPECTS

While in certain laboratory animals and some diseased men the spleen assumes a hematopoietic role in adult life, the average human spleen has little to do with the generation of new blood cells. It does function however as an exquisite filter of the blood, catching within its clutches erythrocytes, granulocytes and platelets. The extravascular flow of these cellular elements through the haphazardly arranged splenic strands is slow, the pH relatively acid and the oxygen tension low. As a consequence, the anucleate and metabolically vulnerable erythrocytes are subjected to severe stresses from which only the most hardy survive. Those that fail are engulfed and degraded by the ever present macrophages. Successful erythrocytes that reach the venous sinuses must pass muster once again as they squeeze between the lining reticuloendothelial cells. During this process aberrant bits of membrane are removed, intracellular inclusions are "pitted out", and a preened and pristine cadre of red cells are returned to the systemic circulation.

In addition, platelets and granulocytes are sequestered within the red pulp of the spleen but can be returned to the circulation upon the application of an appropriate stimulus such as epinephrine or bacterial endotoxin. While the "pore size" of the venous sinuses is adequate to allow platelets easy access to the venous side, the emigration and release of granulocytes occurs by mechanisms that are imperfectly understood.

EFFECTS OF SPLENECTOMY

A classic method of demonstrating the physiologic function of an organ is to extirpate it surgically and observe the consequences. According to Pliny, the Roman naturalist contemporary with Christ, splenectomy was then in vogue as a means of aiding the "wind" of long distance runners (4). There is some doubt to the validity of this assertion and most observers feel that successful splenectomy has been performed in man only within modern times, i. e. the past 100 years. At the dawn of modern surgery, the medical community held its collective breath and contemporary medical journals published reports of follow-up on such patients very carefully. In fact, based on the results of these widely published surgical endeavors, it was gradually and widely believed - and in fact is still believed today - that the spleen is a non-essential organ: one whose functions are diffuse, non-critical, and can readily be assumed by other organs and tissues within the body. To an immunologist who remembers that the thymus was once labeled as non-essential, the notion that the spleen is also redundant is, at the very least, suspect.

Experimental splenectomies performed in animals during the nineteenth century settled several major issues: male dogs minus their spleens still chased females with functional intent; offspring of splenectomized rabbits born with spleens; completely excised spleens do not regenerate, although partial fragments could enlarge. During the first half of this century a variety of claims for abnormalities following splenectomy were made, chiefly in the realm of hematology, but none reached general acceptance.

The hematologic sequela of splenectomy in adult man are perhaps the best documented of the effects of this operative procedure. In the immediate post-operative period one finds elevated platelet counts (often exceeding 1 million per cu mm), elevated granulocyte counts, and changes in the patient's erythrocytes: anisocytosis, poikilocytosis, spherocytes, target cells, Howell-Jolly bodies (retained nuclear fragments). Gradually the platelet and granulocyte counts may return toward normal, but the hemoglobin and hematocrits remain stable, silent testimony to the ability of non-splenic reticuloendothelial tissues to assume this graveyard function off the spleen.

In 1952, King and Schumaker reported a high incidence of fatal bacterial infections in infants following splenectomy (5). While this observation precipitated a debate in clinical immunology and infectious disease that continues up to the present it had the ancillary benefit of reactivating interest in the physiologic functions of the spleen.

IMMUNOLOGIC FUNCTIONS OF THE SPLEEN

Practically one third of the adult spleen is comprised of white pulp: i. e. lymphoid tissue. The presence of lymph follicles with germinal centers affords histologic verification of experimental observations that the spleen is a major source of antibody following antigenic exposure. The anatomical position of the spleen - inserted into the blood vascular circuit - suggests that it functions more or less like a conventional lymph node, except that its affluent is blood rather than lymph. Thus the spleen would be expected to fulfill the role of a giant hemolymph gland, sitting astride the vascular circuit, filtering and entrapping antigens and lymphocytes, thereby providing an ad hoc microenvironment for the cellular interactions with each other and with antigen that form the basis of the primary immune response and lead to the elaboration of specific antibody. That this function for the spleen is partly correct has been amply verified. That this view may be somewhat naive and shortsighted underlies the thesis of this presentation.

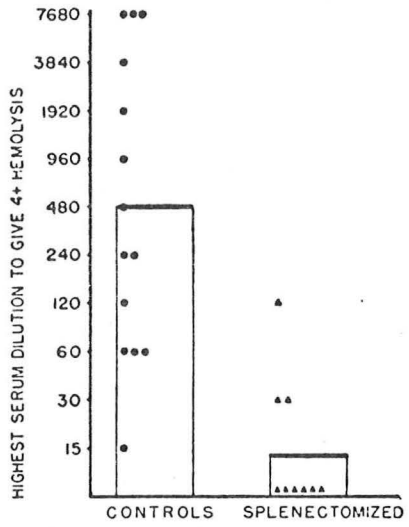
SPLENECTOMY AND PARASITIC INFESTATIONS

In those regions of the globe where parasitic infections, notably malaria and schistosomiasis, are endemic, it was learned subsequent to the first surgical splenectomies, that this organ played a crucial role in the host's ability to cope successfully with his disease (6). Individuals in whom splenectomy has been carried out for traumatic or incidental surgical reasons unrelated to parasitic disease experience a considerably increased risk of malarial infection in the post-operative period. Not only was this incidence of clinical infections increased, but the severity of disease was similarly increased, as was the incidence of death due to malaria. Moreover, in patients in whom a somewhat symbiotic relationship with their malarial parasites had been arrived at naturally, splenectomy brought on a swift and severe recrudescence of the heretofore quiescent infection. By what mechanism(s) does the spleen perform this crucial role in defense against parasitic infections? Is it a function of antibodies produced in the white pulp? Or are the innumerable macrophages and reticuloendothelial cells the major determinants? Whatever the mechanism, it seems clear that the advanced sanitation and nutritional standards of America and Western Europe have submerged from clinical view a role for the spleen that is crucially important in the tropics of Asia, Africa and South America.

IMMUNOLOGIC REACTIVITY AFTER SPLENECTOMY

Approximately 30 years have gone by since immunologists first realized that antibodies are produced by lymphoid cells and their derivatives. Critical evaluation at that time of the relative lymphoid mass of various internal organs suggested that the spleen, by virtue of its major content of lymphocytes, must be important in antibody production. Accordingly, D. A. Rowley (7) carried out a series of elegantly simple experiments in splenectomized animals and man and came to the obvious conclusion that antigens, especially particulate ones, when presented to an individual for the first time by the intravenous route, lead to antibody production which initially comes almost exclusively from the spleen. Antigen delivered by alternative routes elicit antibody responses primarily from the regional, draining lymph nodes. Moreover, in splenectomized individuals, the early antibody response to antigens delivered intravenously is blunted and retarded in comparison to alternate routes of antigen presentation (see Figure 1). However, Rowley then went on to demonstrate that after the

FIGURE 1



14-day hemolysin titers for 14 controls and 9 splenectomized patients

initial interval following immunization by the IV route, antibody production gradually shifted to non-splenic sites. Two - three weeks after immunization via the intravenous route, antibody titers were comparable in splenectomized and sham-operated controls. Almost at the same time the Taliaferros (8) demonstrated that in the primary immune response to intravenously administered sheep erythrocytes in rabbits the spleen provided the initial burst of antibody produced until the peak serum titer was achieved; rapidly thereafter non-splenic sites of specific antibody production intervened and the titer of antibody to sheep RBCs gradually fell. Curiously, in splenectomized animals, responses to secondary antigenic challenge induced much higher hemolysin titers than in animals similarly challenged but with functioning and intact spleens. These early studies pointed clearly to a kind of immunoregulatory role for the spleen, but the cellular basis for the immune response was so poorly understood that the observations went unappreciated.

The clinical impact of these primitive studies on splenic function was quickly realized, and within two years of Rowley's articles, King and Shumacker reported that infants splenectomized for jaundice ostensibly due to hemolysis showed an alarming susceptibility to the development of severe and often lethal infections with encapsulated microorganisms (5). To their initial 6 cases out of 100 splenectomized infants, other authors added additional cases and rapidly the medical community was thrown into a controversy regarding the role of splenectomy in lethal infections by gram positive cocci and H. influenza (9, 10, 11, 12). After the idea of risk of infection had swelled to include all patients that had undergone splenectomy, reason finally prevailed and it is now agreed by those that are au fait with this matter that it is only the newborn or very young child who carries the increased risk of infection post-splenectomy (13). No convincing evidence can be found to substantiate the claims that older children or adults have any increased risk of overwhelming sepsis following splenectomy.

Experimental verification of the validity of these clinical observations came from the work of Shinefield, Steinberg and Kaye who demonstrated that in pathogen free mice, within a narrow dosage range of intravenously delivered Diplococcus Pneumoniae, lethal infections occurred if the animals had previously been splenectomized (14). This susceptibility persisted for approximately 4 months after the operation. Numbers of organisms outside the narrow experimental range, whether delivered intravenously or by other routes, were rarely fatal in splenectomized or normal animals. The net thrust of this and similar data generated during the 1950s and early 1960s can be stated as follows: In those special circumstances where particulate antigens are administered for the first time to an individual by the intravenous route, the spleen can be expected to be the first immunological organ to respond with antibody formation specific for the antigen. Subsequently, antibody production (and the cells that produce it) peripheralizes, and gradually eclipses the splenic role. For all other routes and kinds of antigenic presentation - which must surely constitute the vast majority of instances in our daily lives - the peripheral lymph nodes or gut-associated lymphoid structures provide the initial and sustaining immunologic responses. Not a very glamorous or even significant immunologic role for an organ the size, location and high clinical profile as the spleen!

STRAWS IN THE WIND

A couple of Germans reported in Z. Immunitaetsforsch. in 1963 (15) a rather unexpected finding while skin testing tuberculin sensitive guinea pigs that had been splenectomized. Quite contrary to the anticipated result, splenectomized animals displayed much more intense delayed hypersensitivity reactions to PPD than did their sham-splenectomized confreres. Obviously, the splenectomies had been carried out as a means of suppressing the immune reactivity of these animals and the opposite was found. To make sense out of this paradoxical finding, these workers would have had to have been aware of the elegant and prescient work of Jonathan Uhr, his collaborators, and other contemporary investigators (16), who were only then beginning to dissect out the two effector modalities of the immune response: specifically sensitized lymphocytes and

specific antibody. Uhr showed unequivocally that the induction of the immune response (which he was certain must be based on lymphoid cellular differentiation and proliferation) was critically influenced by antigen and most importantly by specific antibody. The legacy of his observation (that small amounts of antibody delivered shortly before or after initial exposure to antigen could abrogate the primary response to that antigen) is the clinical use of Rogam in the prevention of Rh disease of the newborn. What was crystal clear from this line of investigation was that the induction of the immune response was under some kind of mysterious controlling mechanism(s) which could be readily perturbed by specific antibody or antigen itself. The nature and site of that mechanism remained obscure.

Simultaneously, other workers revealed that the end result of a given immune response depended upon the interplay of antigen and the two effector modalities: antibodies and sensitized cells. In some studies, these modalities appeared to act synergistically leading to the destruction of a target allograft, or tumor, or parasitic organisms; yet, in other experimental designs, antibody appeared to interfere with the destructive capacities of effector lymphocytes thereby protecting from rejection an allograft of kidney or an alien tumor, or the elicitation of an autoimmune disease. Reports of a unique kind of antibody began to appear in the immunologic literature, a tissue protective antibody which was given the designation "enhancing" antibody because it appeared to "enhance" the survival of a tumor graft, obviously to the detriment of the host. Studies by Batchelor (17), Moller (18), Kaliss (19), Snell (20), and others indicated that this special class of antibody only functioned under carefully defined experimental conditions; no general synthesis of the ultimate role of these macromolecules was advanced.

In 1959, at a symposium in Britain on tolerance and enhancement, Richmond Prehn presented some preliminary experimental work which suggested that the spleen was the major, if not the exclusive source, of enhancing antibodies (21). The thrust of his findings was not lost on the interested immunologic community; over the next decade a variety of studies were carried out attempting to verify this role for the spleen. Some of these studies will be considered in more detail later, but it is reasonable to summarize the experience of that decade as follows: while the spleen may very well serve as the major source of enhancing antibodies, the relationship of these antibodies to the clinical problem of renal transplantation and tumor immunity was totally obscure. With the advantage provided by the retrospectoscope, it seems that the difficulties encountered by the many frustrated workers in this area stemmed from a fundamental bias that the prime role for enhancing antibodies would be found at the end of the efferent limb of the immunologic response: that these peculiar antibodies somehow masked or protected in the periphery the target tissue bearing the appropriate antigens. It is the bias of this presentation that enhancing antibodies play a crucial role in many immunologic responses, but not only in the periphery; rather, their major impact is made during the induction of the primary immune response, and especially within that special inductive milieu of the spleen.

THREE IMMUNOLOGIC ENIGMAS

Three immunologic phenomena have been chosen as a means of highlighting the current dilemma in immunoregulation.

Neonatally Induced Tolerance

When Billingham, Brent and Medawar published their now classic paper on the neonatal induction of tolerance to transplantation antigens by the inoculation of allogeneic adult bone marrow cells (22), a major bulwark of support was achieved for the clonal selection hypothesis. This demonstration of prolonged acceptance of grafts bearing alien antigens offered experimental proof of Burnet's prediction that non-self antigens presented to a developing animal prior to self/non-self discrimination would be henceforth regarded by that animal as self. It was thus believed that in this instance tolerance was due to the specific deletion of clones of immunologically active cells with the capacity to recognize these antigens. Recently, however, two lines of experimental evidence have cast doubt on the validity of this interpretation: most notable and notorious has been the Hellstroms' discovery that (1) the serum of animals rendered tolerant neonatally contains "blocking factors" with specificity for the tolerated antigens and (2) lymphoid cells obtained from these animals react in vitro as though specifically sensitized to the same antigens (23). In our laboratory (24) as well as that of others studying fraft-versus-host disease in adult animals, it has been found that a wide variety of antibody forming cells exist whose immunologic specificities are directed predominately at auto-antigens. The dogma of clonal deletion of self-reactive lymphocytes no longer commands the absolute faith of immunologists.

Low Dose Tolerance/Immunologic Deviation

In 1948, Felton described the production of tolerance in adult animals - the immunologist's holy grail (25). Large doses (several milligrams) of poorly degradable antigens (pneumococcal polysaccharide) and massive doses (several grams) of readily catabolizable ones (heterologous serum proteins) could achieve this end. Serendipitously, Dresser (26, 27) discovered in 1962 that tolerance could be achieved in adult animals with much smaller doses (micrograms) of antigen. This surprising finding, which was corroborated by several other laboratories over the next five years (27, 28, 29, 30, 31, 32), was found to be highly dependent upon certain experimental conditions: the antigen had to be soluble, lack inherent pharmacologic properties of an inflammatory nature, and had to be delivered in small quantities by the intravenous route. When animals treated in this manner were subsequently challenged with an immunizing dose of the same antigen in Complete Freund's Adjuvant, they were specifically unable to display delayed hypersensitivity skin reactions to the antigen in question, and they rarely made circulating antibodies that were demonstrable by a double diffusion assay in gel. However, Streilein and Hildreth found that these animals were not immunologically non-reactive to the specific antigen; their serum in fact contained small quantities of a unique class of 7S immunoglobulin which has been termed homocytotropic; that is, it readily fixes to cells of a variety of tissues and upon meeting antigen induces the local release of histamine. Asherson coined the term immunologic deviation to describe this peculiar form of reactivity, but the mechanism of its induction and maintenance were unresolved.

Immunologically Privileged Sites

Allogeneic grafts of skin or kidney when placed orthotopically are rejected with a characterisitc vigor and tempo depending upon the immunogenetic disparity between graft donor and host. There are, however, several anatomically defined sites in which allogeneic grafts can survive for considerable periods of time. These immunologic sanctuaries include the brain, the anterior chamber of the eye, the hamster cheek pouch, and surgically prepared skin pedicle flaps of the flanks of laboratory animals. It has been reliably demonstrated that these diverse sites share one common feature: the absence of a demonstrable lymphatic drainage (33, 34). As a consequence, it is now generally accepted that the immunologic privilege afforded alien grafts placed in these sites turns upon the interruption of the afferent limb of the immunologic reflex arc, preventing the host's immunologic apparatus from perceiving that a foreign tissue is there. Recently, this simple view has been rendered rather non-operative by the demonstration by Kaplan in our laboratory (35), as well as by others (36), that grafts of allogeneic skin or lymphoid cells placed in the anterior chamber of rats or in skin pedicle flaps elicit the production of specific antibodies which can be found in the recipient's serum during the first week after grafting. Since neither the eye nor the skin pedicle flap are organized lymphoid tissues and can not support the in situ production of these antibodies, these findings must mean that the presence of allogeneic antigens within these so-called privileged sites makes an impact on the host's immunologic apparatus sufficient to evoke the systemic production of specific antibody.

It is possible that these seemingly diverse immunologic anomalies have a common mechanistic basis which turns upon a pivotal role assumed by the spleen: that of a biologic transistor.

ABOUT TRANSISTORS

At the outset, I disclaim any definitive understanding of the atomic and electronic basis for the usefulness of transistors. My intellectual curiosity for these remarkable devices has in the past been readily satisfied by my solid state hi-fi and by my trusty desk calculator. In some dim and distant way, the magic of transistors for me has been their capacity to amplify a weak electrical signal many million fold, and to rectify an alternating current into a unidirectional direct one. These tasks are performed by a device which is of very small size, requires a minimum of power, and is of long life (37). The intrigue with regard to the immunologic functions of the spleen vis-a-vis electrical transistors is that a relatively weak signal that is inherently ambivalent (consisting of two alternate forms) can be rectified into a unidirectional response that can be enormously amplified.

CELLULAR BASIS OF THE IMMUNE RESPONSE

Before considering the cellular constitution of the spleen, let me state briefly the current dogma regarding the cellular interactions that form the basis of the mature immunologic response (38)). The presentation of antigen (generally by a macrophage) to a specific antigen reactive thymus dependent lymphocytes triggers a plasma membrane borne receptor to activate that particular T cell. This transforming T cell has two not-necessarily-exclusive options: (1) it may clonally expand in an organized lymphoid tissue and then peripheralize, thus increasing the likelihood that the same antigen will be confronted by specific effector T cells in somatic tissues and elicit a typical

delayed hypersensitivity reaction; or (2) it may interact also within an immunocompetent organ with a B lymphocyte bearing immunoglobulin receptors for the same antigen. As a consequence, the activated B cell clonally expands and elaborates its secretory product, specific antibody. It is known that the same T cell may serve as effector (killer) and helper (39), but the ultimate destiny of a T cell that has entered into a collaborative interaction with a B cell is unknown. Obviously, during the interval of its collaboration it is unable or unavailable to function as an effector cell in the periphery. Moreover, the specific antibody product of the B cell competes more or less successfully in the periphery for the same antigenic determinants to which the effector T cells are drawn. Within the limits of this dogma lie the ingredients for control, amplification and suppression of the immune response.

A PRIMER ON THE SPLEEN

The spleen accounts for but a trivial amount of the total body mass. Its weight is approximately 0.3% of the normal adult human body. Yet, if one determines the relative blood flow through this organ, one finds that it receives approximately 3% of the total cardiac output. On a weight basis (see Table I),

TABLE I

DISTRIBUTION OF CARDIAC OUTPUT TO VARIOUS ORGANS AND TISSUES (% TOTAL)

Skeletal Muscle	XXXXXXXXXXXXXXXXXXXX
Kidney	XXXXXXXXXXXXXXXXXX
Liver (Hepatic Artery & Portal Vein)	XXXXXXXXXXXXXXXXXX
Brain	XXXXXXXXXXXX
Stomach & Intestines	XXXXXXXXXXX
Skin	XXXXXXXXX
Spleen	XXX
Heart	XXX
Lymph Nodes, Peyer's Patches	)

this constitutes more than twice the net blood flow delivered to the myocardium and only the brain, liver and kidney receive a greater relative blood flow (40). Considering the entire lymphoid mass, and excluding the thymus which does not share in the circuit of recirculating lymphocytes, the spleen constitutes less than 1/2 the lymphoid mass, yet it receives approximately 6 times more total blood flow than the rest of the lymphoid mass combined. Thus a disproportionately large share of recirculating lymphocytes, almost exclusively T cells,

as well as intravenously administered antigens, are delivered to the spleen when compared to the rest of the lymphoid complex.

Several recent studies (41, 42, 43, 44) have attempted to determine (a) the relative proportions of T and B lymphocytes in various lymphoid organs and (b) the distribution patterns of T lymphocytes after intravenous inoculation. Tables 2 and 3 represent a compilation of these data. In summary it can be seen that the ratio of T:B cells in the peripheral lymph nodes is 85:15, while in the spleen it is approximately 50:50. The initial fate of lymphocytes injected intravenously into a normal individual is primarily the spleen; subsequently they

TABLE 2

RELATIVE DISTRIBUTION OF T & B LYMPHOCYTES IN RATS (%)

	THYMUS	THORACIC DUCT	LYMPH NODES	SPLEEN	BONE MARROW	BLOOD
T CELLS	99	87	85	50	13	61
B CELLS	<1	10	15	50	40	33

(After Goldschneider & McGregor; Specific anti T and anti B cell rabbit sera used)

TABLE 3

DESTINY OF ^{51}Cr-T LYMPHOCYTES IN ISOGENIC RATS (% TOTAL ^{51}Cr INJECTED)

	THYMUS	LIVER	MESENTERIC LYMPH NODE	SPLEEN	NON-IMMUNOLOGIC ORGANS	BLOOD
@ 4 HRS.	0	6	8.5	35	12.3	1.5
@ 24 HRS.	0	6.7	16.6	18	13.1	2.2

(After Sprent.)

can be found in lymph nodes as well. Over the past 3 - 4 years, experimental models which purport to study lymphocyte interactions have induced a conceptual proliferation of T and B cell types: T cells that help other T cells, T cells that suppress other T cells or B cells (45, 46). In short, the initial complexity introduced by Claman of two classes of lymphocytes which cooperate in the generation of an immune response has been so attractive to obfuscation-seeking immunologists that it has spawned unimagined complexities of sub- and sub-sub classes of cells.

In an attempt to cut through this morass of phenomena and epiphenomena, I would like to take the liberty of quoting from that great logician of the 13th century, William of Occam: "entia non sunt multiplicanda praeter necessitatem" which translates into "beings ought not to be multiplied except out of necessity". A more prosaic statement of this great dictum would be "to account for the events in question, employ a simple hypothesis that requires the least assumptions". Physicians since Osler have applied the famous Occam's Razor to differential diagnosis by striving to employ one diagnosis to explain the entire clinical presentation of a single patient. Applying Occam's Razor to the issue of immunoregulation and the role of the spleen, one can generate a simple mathematical model based on the experimental observations included in the two tables listed above. Among the many things I am not, I am most assuredly not a mathematician. Whatever sense can be made of what I am about to relate can be ascribed to Dr. Campbell Read of the Biometrics Department, who demonstrated an extraordinary ability to grasp my poorly formulated ideas. We have designed a mathematical model that attempts to explain the transistor role of the spleen: it is based on the hypothesis that the movement of any particular T lymphocyte is at random (Brownian motion) unless that cell is confronted with specific antigen. When this confrontation occurs in an environment consisting predominately of macrophages, clonal expansion and differentiation of these antigen reactive T cells into effector T cells ensues; alternatively, when that environment also contains B lymphocytes similarly able to recognize the same antigen, collaborative interactions between these two cell types occur and antibody formation is the result. In refusing to invoke suppressor T cells or a variety of other subclasses of cells, we have made several simplifying assumptions: (a) antigen reactive T lymphocytes recirculate throughout the body in very large numbers and at an enormous rate, while B antigen reactive cells by comparison are essentially sessile; (b) each of these cell types can only respond meaningfully to the particular antigen which each is able to recognize; (c) the only important interactions between a T and a B cell occur with the aid of that particular antigen; (d) T cells entering collaboration with B cells are unable (at least temporarily) to leave the lymphoid organ and rejoin the recirculating pool; (e) the intensity of a cell-mediated effector response in peripheral tissues is a function of the clonal size of antigen-reactive T cells present outside of organized lymphoid organs.

Using the data from Tables 2 & 3 for any antigen (i), the initial distribution of T_i and B_i reactive cells in the spleen and lymph nodes is as follows:

	T_i	B_i
Spleen	$T_{s,i} = 50\ c_i$	$B_{s,i} = 50\ c_i$
Lymph Node	$T_{l,i} = 85\ c'_i$	$B_{l,i} = 15\ c'_i$

where c_i is a constant which depends upon the clonal size of cells reactive with antigen i.

In unit time, suppose the fraction of cardiac output passing through the spleen is θ, and through the lymph nodes (total) is θ'. Suppose further that the number of T_i cells in the spleen at any given moment is $p\ c_i$, and of B_i is $(1-p)c_i$ [so that the relative proportions of T_i and B_i cells are $p:(1-p)$].

Let the corresponding numbers in the lymph nodes be $p'c'_i$ and $(1-p')c'_i$ [so that the relative proportions in the lymph nodes are $p':(1-p')$].

Example: In normal subjects, $p = .50$ and $p' = .85$. Further, $\theta = .03$ and $\theta' = .005$. If the capacity in the spleen is equal to that in the lymph nodes, then $c_i = c'_i$. (1)

Generally, if spleen capacity = k x (lymph node capacity), $c_i = kc'_i$ (1*)

Now we may characterize the number of $T_i \bullet B_i$ matings in the spleen by a Poisson distribution with mean (per unit time)

$$\lambda_i = \theta(pc_i)[(1-p)c_i]\lambda = \theta(1-p)c^2\lambda \qquad (2)$$

This is a reasonable model because the number of lymphoid cells in the body runs into the billions, and the number of matings likely to occur is small by comparison; λ is an unknown parameter.

In lymph nodes, the number of $T_i \bullet B_i$ matings has a Poisson distribution with mean (per unit time)

$$\lambda'_i = \theta'p'(1-p')c'^2_i\lambda \qquad (3)$$

The total number of $T_i \bullet B_i$ matings in the body has an expected value

$$\lambda_i + \lambda'_i = [\theta p(1-p)c^2_i + \theta'p'(1-p')c'^2_i]\lambda \qquad (4)$$

If spleen capacity = lymph node capacity, (1) holds and

$$\lambda_i + \lambda'_i = [\theta p(1-p) + \theta'p'(1-p')]c^2_i\lambda$$

Now suppose that the spleen is removed. We assume that this has no effect on the activity in the lymph nodes so that the total number of matings is the number of matings in the lymph nodes with the parameters unchanged and having expected value λ'_i (Equation 3).

We can now set down an expression which measures the loss in $T_i \bullet B_i$ cell matings due to the removal of the spleen.

This is the ratio

$$R_i = \frac{\text{Expected number of matings with spleen removed}}{\text{Expected number of matings with spleen intact}} \qquad (5)$$

$$= \frac{\lambda'_i}{\lambda_i + \lambda'_i}$$

$$= \frac{\theta'p'(1-p')c'^2_i\lambda}{[\theta p(1-p)c^2_i + \theta'p'(1-p')c^2_i]\lambda}$$

Dividing through by λ:

$$= \frac{\theta' p'(1-p') c'^{2}_{i}}{\theta p(1-p) c^{2}_{i} + \theta' p'(1-p') c'^{2}_{i}} \qquad (6)$$

If spleen capacity = lymph node capacity, $c_i = c'_i$, and the above reduces to

$$R_i = \frac{\theta' p'(1-p')}{\theta p(1-p) + \theta' p'(1-p')}$$

$$= \frac{1}{1 + \dfrac{\theta}{\theta'} \cdot \dfrac{p(1-p)}{p'(1-p')}} \qquad (7)$$

We may note that, even if a Poisson distribution is not the appropriate model, the derivation of (6) and (7) holds for any probability distribution of matings with expected values given by (2) and (3).

Example (for normal subjects) Equation (1) holds, $p = .50 = 1-p$;

$p' = .85$, $1-p' = .15$; $\theta = .03$ and $\theta' = .005$. Equation (7) holds,

and the ratio of expected number of matings is

$$R = \frac{1}{1 + \dfrac{.03}{.005} \cdot \dfrac{.50 \times .50}{.85 \times .15}} = \frac{1}{1 + 11.7647}$$

$$= .07834$$

In effect what this simple mathematical model predicts is that if the assumptions about cellular distribution, conditions for interactions, relative lymphoid organ size and blood flow are reasonably correct, then in an immunologically intact individual, approximately 93% of all T-B cellular interactions elicited by an intravenous inoculation of antigen (i) occur within the spleen, simply because of its unique vascular, architectural and microenvironmental properties.

When this organ is removed, the probability that antigen will elicit collaborative T-B cell interactions is reduced 13-14 fold; instead, antigen induced clonal expansion of T cells within lymph nodes relatively poor in B cells will occur and the systemic expression of the resultant immune response will be dominated by T cell mediated reactivity.

While one need not extend models ad absurditum it would be instructive to consider in this context the additional burden on T cell effector function provided by the antibody products of the B cells with which a T cell collaborates. These antibodies, especially as produced by the cells in the spleen, interfere markedly with the ability of T cells in the periphery to recognize and destroy target tissue bearing the appropriate antigens. However, experimental estimates of the amount of antibodies produced per unit time and their relative binding affinities with antigen are simply not available. Intuitively, one would suspect that the production of specific antibody in conjunction with the collaborative diversion of T cells reduces even further the probability of a specific cell-mediated immune response.

The analogy of the spleen to an electrical transistor is justified by its ability to rectify alternating T cell capabilities (killing versus helping) into predominately helper activity. By channeling that response unidirectionally, a clonal expansion of B cells results and upon differentiation these cells produce logarithmically increasing amounts of specific antibody, thus amplifying enormously the total immunologic response of the host to the inciting antigen. Moreover, if a transistor receives an electrical signal at a particularly appropriate frequency (usually slowly periodic) and intensity (weak), no amplification occurs: instead a "short-circuit" intervenes, the signal is not propagated and in the interval thereafter the instrument cannot handle any signal, irrespective of frequency and intensity. Thus, one might expect that within certain dose ranges, antigen delivered to the spleen at a given frequency should not only fail to induce antibody formation in significant amounts but should render the organ refractory to an immunogenic challenge during the subsequent interval.

If the spleen in fact functions as a biologic transistor either by (a) rectifying an ambivalent cell-mediated/antibody mediated response into a predominant antibody response and at the same time amplifying that response or (b) shorting-out so that a refractory period follows a stimulus of appropriate frequency and intensity, then splenectomy should favor the domination of cell-mediated responses and reduce the possibility of tolerance induction.

RECONSIDERATION OF IMMUNOLOGIC ENIGMAS

Permit me to return to the three immunologic enigmas I related before and describe for you the startling effects splenectomy had in experiments involving each.

Neonatally Induced Tolerance

Neonatal Lewis rats were splenectomized or sham-splenectomized at birth and then inoculated intravenously with a tolerizing dose of (Lewis x BN)F_1 bone marrow cells. 8 weeks later these animals were grafted with (Lewis x BN)F_1 skin (see Figure 2). The majority of sham-splenectomized animals displayed long-lasting tolerance of these grafts; only one quarter of the panel of splenectomized animals displayed any tolerance whatsoever, the majority of animals rejected the (Lewis x BN)F_1 grafts in a brisk fashion. These experiments have recently been reported by Levinson and Silvers (47).

FIGURE 2

INDUCTION OF TOLERANCE IN SPLENECTOMIZED & SHAM-SPLENECTOMIZED NEONATAL RATS

Operation	Highly tol/ no. inocul.	% Highly tol.
Splenectomy		
(A) Complete	4/18	22.2
(B) Incomplete	7/8	87.5
Sham-splectomized	14/26	53.8

An intact spleen apparently plays an important role in permitting the development of neonatal tolerance. In its absence, specific sensitization leading to graft rejection predominates.

Low Dose Tolerance/Immunologic Deviation

Adult guinea pigs were either splenectomized or sham-operated (see Table 4). One month later they received deaggregated bovine gammaglobulin in a regimen designed to elicit low dose tolerance. These animals were then immunized with BGG in Complete Freund's Adjuvant and challenged intracutaneously with PPD and BGG.

TABLE 4

EXPERIMENTAL PROTOCOL: LOW DOSE TOLERANCE

Day 0: 500 ug BGG IV

Day 7: 500 ug BGG IV

Day 33: 1 ug BGG with CFA in Foot Pad

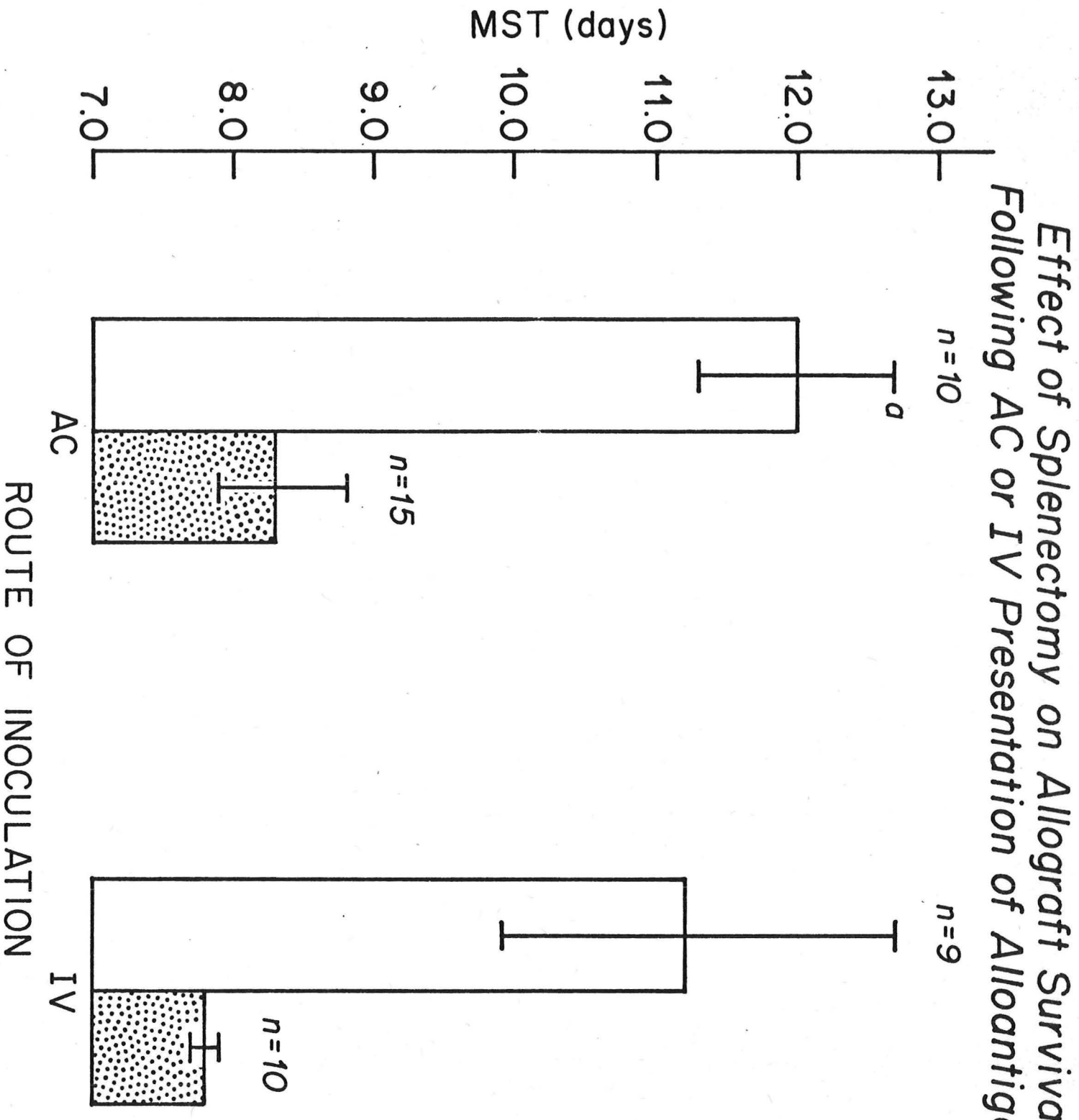
Day 43: Intracutaneous challenge, with BGG & PPD
Serum obtained for anti-BGG precipitins

TABLE 5

RESULTS OF LOW-DOSE TOLERANCE AFTER SPLENECTOMY

EXPERIMENTAL GROUP	ANTIGEN PRETREATMENT	SPLENECTOMY	SKIN TEST BGG	SKIN TEST PPD	PRECIPITINS IN SERUM
I (Control)	No	No	+++	+++	+++
II	Yes	No (Sham)	0	+++	0
III	Yes	Yes	+++	+++	0

FIGURE 3


Effect of Splenectomy on Allograft Survival
Following AC or IV Presentation of Alloantigens
MST (days)
7.0
8.0
9.0
10.0
11.0
12.0
13.0
n=10
a
n=15
AC
n=9
n=10
IV
ROUTE OF INOCULATION

As can be seen in Table 5, none of the sham splenectomized animals showed any evidence of delayed hypersensitivity to BGG, although they reacted vigorously to PPD. Alternatively, the splenectomized animals mounted vigorous delayed reactions to both PPD and BGG. Neither sham nor splenectomized animals receiving a tolerizing dose of antigen responded with circulating precipitins (48).

Clearly, the capacity of an animal to display low dose tolerance (immune deviation) requires the presence of a functioning spleen. In its absence, putative tolerizing doses of antigen followed by typical immunizing procedures elicit vigorous cell-mediated immunity, but no precipitating antibody.

Immunologically Privileged Sites

Fischer rats were splenectomized and then inoculated into the anterior chamber of the eye with (Fischer x DA)F_1 lymph node cells. 10 days later each received a graft of (F x DA)F_1 skin orthotopically. The data presented in Figure 3 indicate the median survival time of these grafts on sham-splenectomized recipients was 12 days, whereas the MST of similar grafts on splenectomized hosts was 8.2 days (see Figure 3 on next page). When one compares these figures with the results of conventional first and second set grafts from (F x DA)F_1 donors placed on normal Fischer hosts, one can only conclude that antigen presented to a naive host via the anterior chamber leads to an alteration of the animals immune response such that a subsequent graft of identical genotype enjoys a prolonged survival. This alteration in responsiveness is totally dependent upon an intact spleen, for when that organ is removed, the animal is committed from the very beginning to a typical cell-mediated response and destroys a subsequent graft in an accelerated manner (49).

As though to underline the role of the spleen in immunoregulation, at a recent faculty seminar Dr. George Mackaness reported that the immunologic response of mice to sheep erythrocytes was materially influenced by the route and amount of red cells presented to the host (50, 51). The appearance of antibody specific for sheep RBCs effectively suppressed and terminated the initial cell-mediated response. The elaboration of that antibody required an intact spleen. In splenectomized animals the primitive and early cell-mediated response continued unabated and undiminished for an interval considerably longer than in animals with functioning spleens.

CLINICAL IMPLICATIONS/PREDICTIONS

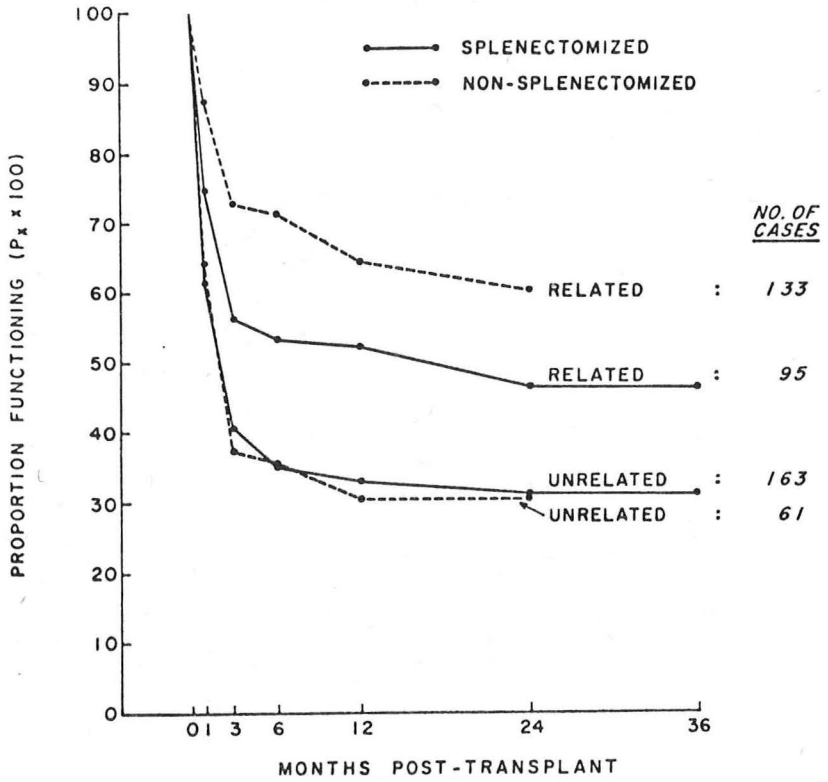
If the spleen does in fact function as a biologic transistor, as these experiments seem to suggest, then there are certain clinical expectations that ought to follow. I shall consider only three areas, although others come to mind: renal transplantation, autoimmune disease, and malignancy.

Renal Transplantation

During those halcyon days of renal transplantation when immunosuppression was hard to achieve, every attempt was made to render the potential transplant recipient as immunodeficient as possible. Reasonable and unreasonable means were

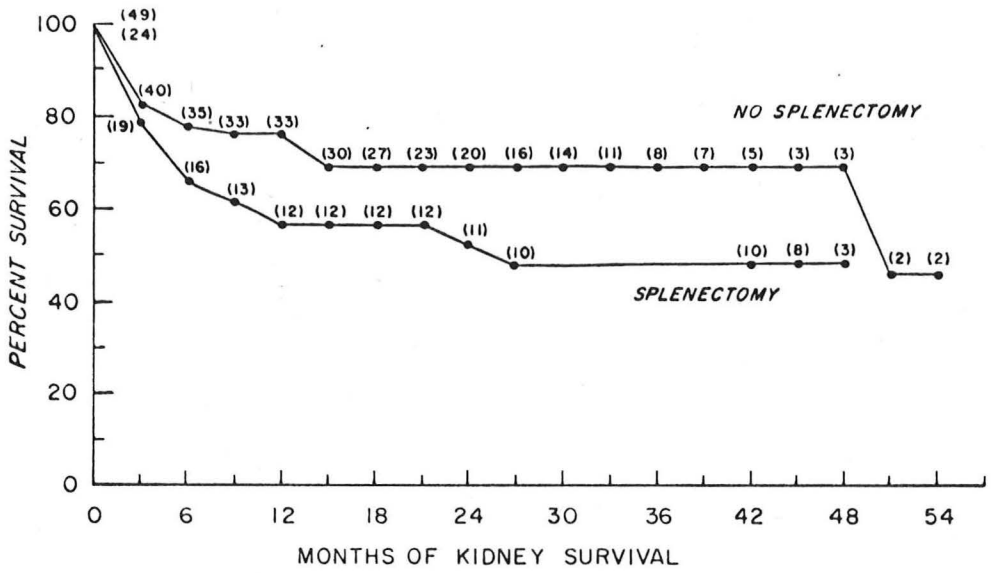
employed including thymectomy and splenectomy before or at the time of surgery. After an ill-fated early report that splenectomy had a favorable influence on the post-operative course of the renal transplant recipient, three definitive reports all came to the same dismal conclusion (52, 53, 54): splenectomy does not provide any additional immunosuppression than that which can be obtained from currently available immunosuppressive drug regimens. It is somewhat instructive to review the data presented in these three papers. The Opelz and Terasaki report indicates that patients with evidence of prior sensitization to transplantation antigens due to transfusions (their sera contained lymphocytotoxic antibodies), whose spleens were removed before transplantation, had a somewhat more rocky course in the interval between 3 and 12 months and more grafts were rejected by these patients during this time than by non-splenectomized patients whose sera also contained lymphocytoxins. Gleason and Murray indicate that among related donor-recipient pairs, splenectomized patients had considerably more rejections during the 3-6 month interval than did their non-splenectomized compatriots (Figure 4). Once again, Figure 5, taken from Pierce and Hume's article, reveals that the percent survival of first renal transplants in recipients from living related donors was considerably worse in the splenectomized group. If the spleen functions to deflect cell-mediated immune responses into antibody-mediated ones, then to the extent that "enhancing" antibodies provide a kidney allograft with some protection from the killer T cells, it might have been predicted that splenectomy prior to transplantation would adversely effect the survival of the graft. Experimental verification of this clinical observation has been reported recently

FIGURE 4



Estimated proportion of transplants functioning (P_x) in splenectomized and nonsplectomized recipients at various intervals post-transplant according to genetic relationship of kidney donor. Note: Proportion of transplants expected to function up to 1, 3, ... 36 months post-transplant.

FIGURE 5



Functional survival of first transplant in recipients of kidneys from living related donors. Each figure in parentheses is the number of patients at risk during the interval after transplantation.

by Enomoto and Lucas who showed that splenectomy carried out 1 week before placing renal allografts into nephrectomized recipient rats abolished immunological enhancement (55). Moreover, Veith, Luck and Murray demonstrated in dogs that the prolonged renal allograft survival which was purchased by immunosuppressive drugs such as Imuran and Azaserine was abrogated by prior splenectomy of the host (56).

Autoimmune Disease

Among the clinical disorders that are considered autoimmune in pathogenesis, only a few are felt to be procured by specifically sensitized lymphocytes; the majority are antibody mediated - Autoimmune Hemolytic Anemia, Idiopathic Thrombocytopenic Purpura, Goodpasture's Syndrome. Based primarily on experimental evidence from laboratory animals, there is good reason to believe that autoimmune thyroiditis may be cell-mediated. Experimental allergic encephalomyelitis is another striking example of a cell-mediated auto-destructive disorder; in fact, the elaborated specific antibody can abrogate or even prevent the paralytic disease. However, a clinical counterpart to this disease has not been reliably identified. A cogent if controversial argument can be developed that cell-mediated autoimmune disorders in man and animals are constantly held in check by the continual protection of self by "enhancing" antibodies or blocking factors. If adequate criteria existed for documenting cell-mediated autoimmune diseases in man, then one would predict that patients whose spleens had been removed for traumatic or incidental surgical reasons would exhibit in the follow-up interval an increased incidence of these diseases. No such data is available

to my knowledge in the literature. But in the laboratory, support for this expectation is incontrovertible. In our own laboratory, one strain of inbred rats was found to be very resistent to the development of allergic thyroiditis irrespective of the immunization schedule employed. Yet, in a panel of splenectomized rats of the same strain, an extract of thyroglobulin inoculated with Complete Freund's Adjuvant produced evidence of severe thyroiditis in most animals at 30 days post-immunization (60 days post-splenectomy) (57). Fortuitously, a group of investigators in St. Louis decided to study the induction of autoimmune thyroiditis in monkeys (58). They set out to reduce the incidence and severity of the disease by splenectomizing their animals before induction of the disease. To their amazement, after a single immunization procedure in splenectomized animals, a severe and fulminant thyroiditis was evoked leading to virtual obliteration of thyroid follicles. By contrast, animals with intact spleens developed non-fibrotic and much less severe lesions despite being repeatedly subjected to similar immunization procedures over a 340 day interval.

Malignancy and Metastatic Disease

Contemporary dogma regarding the relationship of immune responses to malignancy dictates that cellular immunity is capable of destroying neoplastic tissues which bear tumor specific antigens. Moreover, blocking factors or enhancing antibodies directed at these same antigenic determinates interfere with this destructive process. Should this dogma be correct then one might expect that among patients who had been splenectomized for reasons unrelated to malignant disease one ought to see a significantly reduced incidence of malignant disease compared to non-splenectomized controls, because the "transistor" that is crucial to the development of enhancing antibodies and blocking factors is no longer present and unimpeded cell mediated immunity could prevail to destroy emerging neoplastic cells. To place this prediction in an appropriate context, let me briefly review some experimental data bearing on this issue. As already mentioned, Richmond Prehn reported that antibodies capable of enhancing tumor growth were produced primarily within the spleen. During the 15 years since that signal observation, a welter of experiments concerned with it have been carried out. There is now ample evidence to show that in laboratory animals splenectomy (1) reduces the incidence of tumor development in animals exposed to a carcinogen (59), (2) facilitates rejection of tumors induced by Moloney sarcoma virus (60), (3) increases the incidence of spontaneous regression of a murine sarcoma (61), (4) decreases the production of humoral isoantibodies and inhibits the growth of nonspecific tumor sublines (62); (5) that the spleen is essential for the production of tumor enhancing antibodies (63), (6) that the spleen produces this class of antibody only during the first week after tumor graft inoculation, after which non-splenic lymphoid tissues assume this role (64, 65).

In order to determine the post-splenectomy incidence of malignancy in man, it was necessary to seek out the data *de novo* since no such question has heretofore been addressed in the literature. Buoyed by Dr. Eugene Frenkel's anecdotal recollection that in his vast clinical experience he could not remember ever treating a patient with cancer whose spleen had previously been removed, we, *i. e.* Mr. Robert Schoenvogel, a fourth year medical student, and I, began the tedious process of screening the Parkland Hospital medical records of all patients that had undergone splenectomy for traumatic or incidental surgical reasons during the interval 1955-1960. It was felt that this should provide a sufficiently long

follow-up period for a meaningful assessment of tumor incidence to be made. For a variety of reasons adequate follow-up records were available in only 60 of the more than 150 patients identified. By mail and telephone we are currently attempting to trace the rest of these patients. In the meantime, each of the 60 records was scrutinized for evidence of the appearance of malignancy; the number of years of follow-up was recorded. From the tables provided by the National Cancer Institute (66) an age specific incidence was obtained for the age of each patient at the time of splenectomy and at the last follow-up visit. A crude estimate of the risk for developing malignancy for that patient during the follow-up interval was obtained by finding the arithmatic difference between the two age-specific incidences. A cumulative estimate of the risk of developing malignancy for a normal population passing through the same aging intervals was determined. This number served as the expected control rate of tumor incidence, and was compared with the incidence of malignancies observed in this group of patients after splenectomy. In all of the patients, records were discarded from consideration if the reason for the splenectomy was an antibody mediated autoimmune disorder, or if the patient had cancer at the time of surgery. The results of this retrospective study are presented in Table 6.

TABLE 6

DEVELOPMENT OF MALIGNANCY FOLLOWING TRAUMATIC SPLENECTOMY

	Parkland 1955-1960	Parkland 1960-1965	Ek & Rayner	Pederson	Summary
No. of Patients	26	34	18	41	119
Mean Age at Splenectomy	31.3	39	16	23.9	
Mean Follow-up Interval (yrs)	16.7	10.6	14	18.6	
Total Follow-up in Patient Years	347	335	258	764	1704
Expected Incidence of Malignancy*	2.08	2.85	0.3	4.7	9.93
Observed Incidence of Malignancy	1	2	0	0	3

*Based on Third National Cancer Survey, Department of Health, Education and Welfare, NCI, NIH.

While they do not address themselves specifically to the issue of tumor development, there are two studies in the Scandanavian literature describing relatively long term follow-up of patients whose spleens were removed for traumatic reasons (67, 68). Because of the excellent record keeping commonly found in countries with socialized medical systems, very few patients were lost to follow-up. Using the data reported in these two extensive retrospective studies, similar methods of determining risk of tumor development were used to arrive at a predicted incidence of malignancy for a comparable human population with intact spleens. The results of these computations are also listed in Table 6.

By gathering all the data together, a cumulative follow-up interval of approximately 1700 patient years was obtained. While the average patient follow-up interval is 16 years, the range extends from a few years to 40! Based on computations of the expected age-specific incidences, 9.93 cases of malignancy should have developed in this population during this follow-up interval. In fact, only 3 cases of malignant disease were seen. In one of these an incidental, well circumscribed renal cell carcinoma was found at autopsy in a patient who had died of non-malignant disease. The second was a prostatic carcinoma completely excised by TUR with no recurrence three years later. The third patient developed a florid multiple myeloma 6 years after splenectomy and this was the major factor in his demise. In reviewing the autopsy report of one additional patient an incidental prostatic carcinoma was found. Interestingly, an enlarged splenic remnant was also found in the adhesions surrounding the stomach! With the exception of the malignant plasma cell dyscrasia, not a single instance of metastatic disease was found. The incidence of malignant tumors in the splenectomized patients was less than 1/3 the expected incidence for a normal population of comparable age and follow-up interval.

While this finding is in general agreement with the thesis advanced in this presentation, this study of long term effects of splenectomy is still in progress. But it is already tantalizing and irresistable to contemplate the therapeutic implications. With the number of asymptomatic gallstones still at large in the human population, it would be unreasonable to ask our surgical colleagues to take on the awesome task of splenectomizing all adults prophylactically as a means of preventing cancer. More reasonably, however, one wonders whether in selected instances splenectomy might not play an important role in an immunotherapeutic regimen for a patient with an established neoplasm. If the transistor function of the spleen is exerted predominately on antigens gaining access to the systemic circulation, then one might consider that patients with localized tumors or with only local invasion, whose tumor has only begun to shed its tumor specific antigen bearing cells and cell fragments into the peripheral blood, would most likely benefit by splenectomy. The major inductive source of enhancing antibody production would be removed, and the likelihood would be increased that the patient's native cell-mediated immune capabilities could overcome the neoplastic growth. Whether splenectomy might similarly benefit patients with advanced metastatic disease is another matter. Since the cells responsible for producing enhancing antibodies disseminate from the spleen following the induction of the response, removal of the spleen at that point might only further embarrass an individual already debilitated by his disease. With cautious optimism, I submit that the matter of splenectomy for certain patients with malignant disease should at least be considered.

EPILOGUE

If the spleen functions immunologically in a manner analogous to an electrical transistor, as suggested in this presentation, the tremendous evolutionary advantage of such a mechanism for increasingly complex higher organisms must be considered. The validity of Burnet's hypothesis concerning clonal deletion and selection remains unchallenged (69); self/non-self discrimination, an immunologic phenomenon inextricably linked to the thymus and thymic function, is shared not only by all vertebrates, but even by annelid worms and echinoderms (71). It must therefore be a rather primitive facility, one that appeared early in the evolution of multicellular creatures. Alternatively, the ability to produce antibody in response to foreign antigens has emerged at a later time phylogenetically: only in primitive vertebrates (70). The simultaneous appearance in evolutionary terms of the spleen suggests that the two events are closely related (2). It is reasonable to suggest that the spleen provides a milieu in which recirculating T lymphocytes can be captured and enforced into collaborative interactions with antibody-forming B lymphocytes. This phylogenetic milestone has two important consequences: (1) it allows for the amplification of immunologic capabilities so that invading pathogenic organisms can be dealt with more effectively, and (2) it provides for an ongoing immunologic mechanism that ensures that the "neoantigenic" protein products of genes acting in development and differentiation but which are turned on subsequent to the discrimination of self from non-self will be tolerated as self through immunologic enhancement. However, as with all evolutionary gains, these advantages have been purchased at a formidable price: the spleen, in its effort to preserve that which is the differentiated and mature self, fails to perceive the neo-antigens of neoplastic tissues as non-self, and thus enhances their survival as well.

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