

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor***ANTIGEN LOCALIZATION
AND MIGRATION
IN IMMUNITY AND TOLERANCE**THOMAS E. STARZL, M.D., Ph.D.,
AND ROLF M. ZINKERNAGEL, M.D.

SURVIVAL in a hostile environment requires the ability to mount a protective immune response while avoiding a reaction of the immune system against the self. We propose that the migration and localization of antigen are the governing factors in immunologic responsiveness or unresponsiveness against infections, tumors, and self and against xenografts and allografts. This conclusion is based largely on studies of experimental viral infection¹⁻³ and of the small numbers of donor leukocytes found in the blood and tissues of human and animal recipients of organ allografts (microchimerism).⁴⁻⁶ Under both circumstances, an immune response can be construed as a balance between potentially reactive lymphocytes and the composition, quantity, kinetics, and distribution of antigen (foreign or self) in the host.

**CHARACTERISTICS OF THE IMMUNE
RESPONSE**

The immune system reacts similarly against lethal cytopathic microorganisms and less dangerous noncytopathic ones, but with different consequences.^{1,2,7,8}

Immune Responses against Microorganisms***Cytopathic Microorganisms***

In order to prevent damage to host cells by cytopathic microorganisms, the full resources, first of the nonspecific (innate) and then of the specific (adaptive) immune system, are mobilized to eliminate the

damaging microorganisms quickly and completely, without regard for immune-mediated destruction of host tissues.¹⁻³ The first line of defense is dominated by interferons, macrophages, γ/δ T cells, and natural killer cells. In addition, infectious invaders with densely arranged and ordered repetitive epitopes, or those containing lipopolysaccharides, can induce B cells to respond and secrete antibodies without T-cell help. Nonspecific or less specific effector mechanisms, such as complement, interleukins, and phagocytes, are also involved. The specific T- and B-cell immune responses then usually control cytopathic microorganisms definitively.

Noncytopathic Microorganisms

In contrast to cytopathic microorganisms, less cytopathic or noncytopathic microorganisms can be accommodated in ways that allow the host and pathogen to coexist. Intracellular microorganisms are primarily controlled by interleukin-releasing or cytolytic T cells that recognize host cells displaying complexes composed of major-histocompatibility-complex (MHC) molecules plus peptides derived from the infecting microorganism.^{9,10} Because the microorganisms may be noninjurious, the immune response to them should not be one that causes damage to either normal or infected tissue. Otherwise, the immune responses to and destruction of widely disseminated microorganisms (e.g., hepatitis B or C virus) might disable or even kill the host. To prevent this, the immune response can be tempered or terminated by mechanisms that may lead to various degrees of antigen-specific nonreactivity.

Immune Reactions against Transplants***Xenografts***

The mechanisms of predominantly innate immunity, including complement-dependent cell destruction and others based on cross-reactive natural antibodies, are responsible for the hyperacute rejection of xenografts. The best-characterized target antigen on the cells of discordant xenografts is the surface carbohydrate epitope galactose $\alpha(1-3)$ galactose. Since $\alpha(1-3)$ galactose or similar substances are also found on numerous bacteria, protozoa, and viruses,¹¹ they are likely to be responsible for the preexisting natural antibodies.

Allografts

In a prophetic early review, Lawrence¹² compared the stepwise rejection of primary allografts to infections associated with delayed hypersensitivity (e.g., tuberculosis). The MHC-restricted mechanisms of

From the Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh (T.E.S.); and the Institut für Experimentelle Immunologie, Universität Zürich, Zürich, Switzerland (R.M.Z.). Address reprint requests to Dr. Starzl at Palk Clinic 5C, 3601 Fifth Ave., Pittsburgh, PA 15213.

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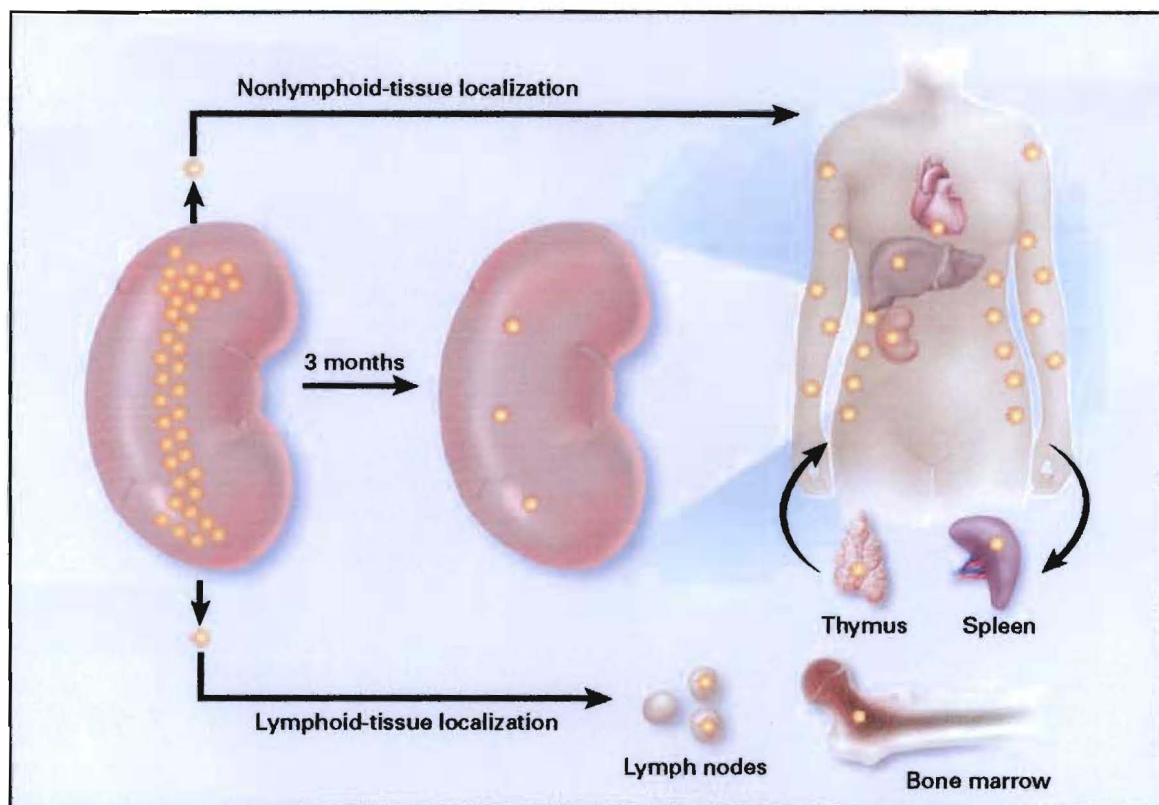


Figure 1. Distribution of Donor Leukocytes (Circles) Three Months after Kidney Transplantation.

With the nearly complete disappearance from the allograft of donor leukocytes, the absence of which reduces the organ's immunogenicity, the recipient's leukocytes replace them. The appearance of chimerism is visible in the nonlymphoid organs (skin, heart, and liver are shown), as is the communication between the nonlymphoid compartments and lymphoid compartments (spleen, thymus, lymph nodes, and bone marrow).

the delayed hypersensitivity response were unknown at the time. Once they were recognized, it was obvious that immune rejection of allografts was the physiologic equivalent of rejection of infected cells.¹⁰

MIGRATORY ROUTES OF ANTIGEN

Because lymphoid organs are critical sites for induction of the response against all antigens, the resulting immune reactions can be correlated with both the routes of spread and the eventual localization of antigen.¹³

Microorganisms

The tissue tropism and pathogenicity of microorganisms determine their migratory routes and destinations. Many infectious microorganisms, particularly of the cytopathic variety, or their component antigens, are transported within a few hours or days to the lymphoid organs, where a protective immune response is quickly induced. Others, especially those with few or no cytopathic qualities, may stay in im-

munoprotected nonlymphoid locations; for example, papillomaviruses may stay in keratinocytes or rabies virus in neurons. Some intracellular microorganisms may take up residence in nonlymphoid sites after surviving for a short time in immune-competent lymphocytes (e.g., Epstein-Barr virus in B cells and human immunodeficiency virus [HIV] in CD4 T cells) or after partially bypassing the lymphoid organs during a generalized infection (e.g., the common hepatitis viruses). This is often achieved by viruses in latent infections in which viral antigens are not expressed by the nonlymphoid cells or are not presented by the cells' MHC molecules (e.g., HIV in some lymphocytes). Alternatively, viral antigens may be expressed in cells that are difficult to reach by immune cells or antibodies (e.g., HIV in brain cells or cytomegalovirus in renal tubular cells).

Allografts

In the first few days after organ transplantation, multilineage, bone marrow-derived ("passenger")

leukocytes of donor origin constitute 1 to 20 percent of the circulating mononuclear cells in the recipient. The percentage depends on the organ that was transplanted; it is highest with liver and intestine and lowest with heart and kidney.⁵ These leukocytes, which include pluripotent stem cells^{14,15} and dendritic cells,^{4-6,16-19} travel to the recipient's lymphoid organs^{16,17,20} and are largely replaced in the graft by similar cells of the recipient.^{5,18,19,21} After about two weeks, increasing numbers of donor leukocytes can be detected in other tissues,^{18,19,22} and by three months they are found mostly in nonlymphoid tissues (e.g., skin and heart).^{5,6,23} After two to four weeks, these leukocytes can be identified in blood or tissue samples only with sensitive immunocytochemical techniques or with the polymerase chain reaction. The detection limit of these techniques is 1 donor cell per 10⁵ recipient cells.^{4-6,23} The kinetics and the eventual patchy distribution of the donor cells resemble those of spreading infections (Fig. 1).²⁴

MECHANISMS OF IMMUNOLOGIC NONREACTIVITY

In the earliest studies of specific immunologic nonreactivity, lymphocytic choriomeningitis virus was found to persist after transplacental infection of the embryo from the mother or after injection of the virus into newborn mice, but it was eliminated immunologically in mice infected as adults.²⁵ Immunologic tolerance to allogeneic blood cells was first reported in nonidentical twin calves with a common placental circulation that were mutually chimeric and therefore reciprocally tolerant.²⁶ Subsequently, experiments in which allogeneic donor splenocytes were infused into mice during fetal or early neonatal life found a strong correlation between engraftment of the donor leukocytes (chimerism) and subsequent acceptance of skin grafts from the donor strain (acquired tolerance).²⁷

In rabbits given bovine serum albumin while also being given mercaptopurine, the cytotoxic drug rendered the animals tolerant of the foreign protein.²⁸ Similarly, manipulation of the immune response with mixtures of nonspecific immunosuppressive drugs administered at the time of organ allotransplantation reliably induced incomplete donor-specific tolerance,²⁹ providing a practical basis for clinical organ transplantation. The mechanisms by which these various nonreactive states evolve have been controversial.

Tolerance Induced by Clonal Deletion

When relatively small numbers of T cells are confronted with an excess of antigen, they disappear (deletion). This phenomenon was long suspected to result from overstimulation of a subgroup of immune cells by a variety of antigens,^{28,30,31} including allogeneic cells.³² Since 1990, the process, called clonal exhaustion or exhaustion-deletion, has been

induced by infection with noncytopathic viruses, by retroviral superantigens, and by the injection of peptides in varying doses without or with adjuvant or of cells expressing a defined foreign antigen (e.g., male H-Y antigen or bacterial superantigen).^{8,33-36} The T cells that react against the specific antigen are activated within a few days, end-differentiate to effector cells, and are deleted.^{1,37,38} The details are poorly understood, but cell death by interleukin deprivation and other mechanisms associated with apoptosis seems to be involved.^{39,40}

Although thymic (central) deletion is the most effective way to eliminate maturing self-reactive T cells in ontogeny and early in the life of many higher vertebrates,⁴¹ efficient purging of T cells (and possibly also B cells) also occurs in the peripheral lymphoid organs,⁴² particularly after transplantation.⁴³ The greater the amount of antigen, and the lower the number of available immune-cell precursors, the more rapid and efficient the establishment of unresponsiveness.

Noncytopathic Microorganisms

Because cytopathic viruses are so rapidly lethal, clonal deletion of T cells can be demonstrated only by infecting animals with noncytopathic viruses. Infection of adult mice with low doses of the noncytopathic lymphocytic choriomeningitis virus leads to sustained development of cytotoxic T cells that eliminate the virus, whereas high doses of virus lead to rapid immune activation, followed by deletion of the virus-specific T cells and viral persistence (tolerance).³⁴

Allografts

Clonal exhaustion-deletion was invoked more than three decades ago to explain the observation that after experimental or clinical organ transplantation, the host-graft relation occasionally was so altered that immunosuppressive therapy could be discontinued.⁴⁴ The hypothesis was not widely accepted, because of the poorly understood role of passenger leukocytes. These donor leukocytes make up the principal immunogenic component of histoincompatible allografts,^{45,46} an effect that has been attributed by some to their expression of MHC class II or costimulatory molecules such as B7.^{8,47}

The rapid disappearance of donor leukocytes from successfully transplanted tissues and organs^{21,45,46,48,49} was long thought to be caused by their selective destruction by the recipient's immune system. After it had been demonstrated that the passenger leukocytes migrated to host lymphoid organs,^{16,17,20} the immune destruction was viewed as occurring both peripherally and within the graft. Allograft acceptance was therefore explained by immune elimination of passenger leukocytes in combination with a panoply of other factors, including the appearance of

veto, suppressor, or other immune-regulatory cells, changes in cytokine profile, and production of antibodies and idiotype networks. It has also been suggested that T cells, both in lymphoid organs and in the periphery, may be turned off ("anergized") if they encounter appropriately presented antigen in the absence of a second signal provided by costimulatory molecules.^{8,47}

Contrary to these hypotheses, we have ascribed the ability of donor leukocytes to induce both immune responses and tolerance mainly to their capacity to migrate to and persist in lymphoid organs.^{1-5,43} With the discovery of donor leukocytes in lymphoid organs and elsewhere in recipients of organ allografts many years after transplantation,⁴⁻⁶ it could be suggested that allograft acceptance involves what has been described elsewhere as widespread "responses of co-existing donor and recipient immune cells, each to the other, causing reciprocal clonal expansion, followed by peripheral clonal deletion."⁴ Clonal exhaustion-deletion of the host-versus-graft response has been demonstrated in rats after liver transplantation.⁵⁰

However, the early exhaustion is usually incomplete or reversible. Low-level anti-graft^{18,22,51} or graft-versus-host⁵¹ reactivity can be detected by *in vitro* assays in cells from many recipients of long-surviving organ allografts. The implication is that a spectrum of host-versus-graft and graft-versus-host reactions may exist in equilibrium in a transplant recipient, ranging from undetectable host-versus-graft as well as graft-versus-host responses at one extreme to a pure host-versus-graft response (rejection) at the other.

This hypothesis is consistent with the historical argument that acquired tolerance is a dynamic, antigen-dependent state^{52,53} whose outcome is strongly influenced both by the dose of donor leukocytes and the strength of the host immune response. Donor as well as recipient precursor dendritic cells, pluripotent stem cells, and committed cells at various stages of differentiation can be found in the lymphoid organs of normal mice that spontaneously tolerate liver allografts.⁵⁴ Under similar experimental conditions, the number of donor cells in mouse recipients of cardiac allografts is far fewer, and they disappear at the same time as the allograft is rejected.⁵⁴

Indifference of the Immune System

Antigens that do not enter organized lymphoid tissue do not induce an immune response. This has been termed "immune indifference."⁷

Noncytopathic Microorganisms

In addition to, or instead of, clonal exhaustion-deletion, early localization in nonlymphoid sites may be necessary for the long-term survival of microorganisms. Even after apparently complete elimination, some infectious microorganisms may ultimate-

ly become inaccessible, in peripheral nonlymphoid sites, to memory cytotoxic T lymphocytes or neutralizing antibodies (e.g., papillomaviruses harbored by keratinocytes).¹⁻³

Allografts

Induction and maintenance of a T-cell response can be prevented by removal of donor leukocytes from thyroid^{55,56} or other histoincompatible allografts.^{45,46,48,49} Donor-specific tolerance does not evolve, however, and rejection can be precipitated by an immunizing injection of donor leukocytes.^{46,55,56} Many similar experiments have confirmed that immune reactivity is induced only in lymphoid organs to which alloantigen-expressing donor cells can move directly.

The importance of alloantigens that are processed in and presented with MHC class II molecules by host antigen-presenting cells is unclear.⁵⁷ Taken collectively, however, the evidence suggests that if the cells from allografts do not reach lymphoid organs, a specific cytotoxic T-cell response either is not induced or cannot be maintained.

CLINICAL CORRELATIONS

The classic host-versus-graft paradigm has provided a useful context for *in vitro* studies of immune function (e.g., mixed-lymphocyte-culture assays). However, the effects of live antigen can usually be better understood through experiments in animals or clinical observations, particularly if the cells expressing antigen include immunocompetent lymphocytes.

Noncytopathic Microorganisms

As a general rule, the immune response to acute microbial infections is a one-way immune reaction (i.e., antimicroorganism).¹⁻³ The survival of some microorganisms may be facilitated by their ability to release cytokines ("virokines"), imitate cytokine receptors (e.g., poxviruses), induce products that interact with complement components (herpesviruses) or factors modulating MHC class I molecules (adenoviruses), or modify transcription factors.^{58,59} Lymphocytic choriomeningitis virus or HIV can even cause immunopathologic manifestations resembling graft-versus-host disease or lead to immunosuppression that may help the virus to persist.⁶⁰

Infection with the hepatitis B and C viruses and probably other widely disseminated noncytopathic microorganisms, such as lymphocytic choriomeningitis virus in mice or HIV in humans, can induce the entire spectrum of responses from clonal activation to deletion. At one extreme, clonal deletion may correlate clinically with an asymptomatic carrier state in which immune reactivity is either zero or minimal relative to an excessive antigen load (Fig. 2A). Alternatively, the early induction of effector T cells may eliminate detectable viruses rapidly, with resulting long-term protective immunity (Fig. 2B).

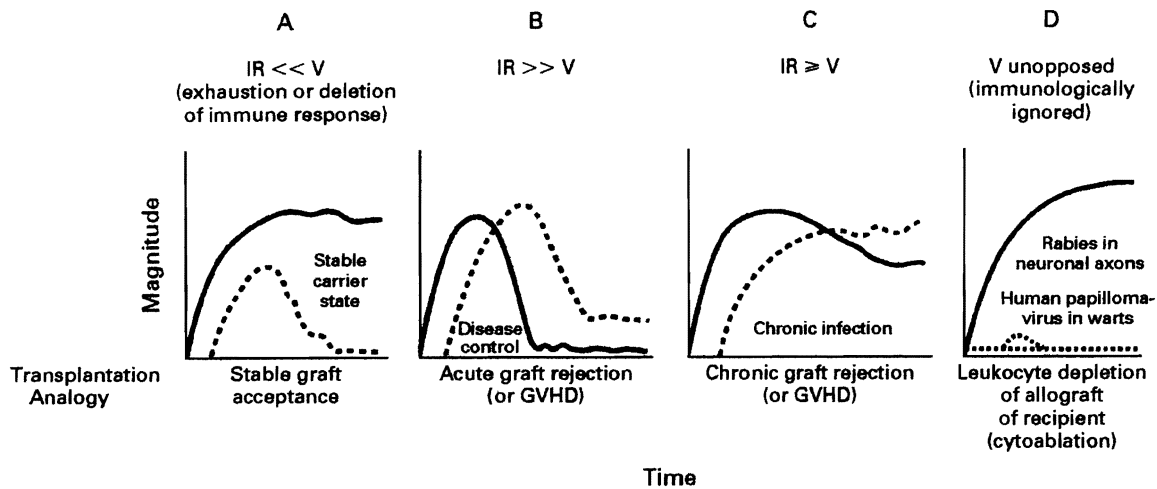


Figure 2. Potential Outcomes after Infection with Noncytopathic Microorganisms and Analogies (Expressed as Rejection or Graft-versus-Host Disease) to Organ and Bone Marrow Transplantation.

The horizontal axis denotes time. The vertical axis shows the magnitude of the viral load (V, solid line) and the host immune response (IR, dashed line). In Panel D, the IR is absent (straight dashed line) or minimal. GVHD denotes graft-versus-host disease.

Some of these apparently eliminated infections may never be completely cleared.^{2,24} Viruses may persist in peripheral nonlymphoid tissues (e.g., hepatitis B and C viruses and lymphocytic choriomeningitis virus in the hepatocytes and cytomegalovirus in the renal tubules), where they may subsequently be ignored. Leakage of small quantities of hidden antigen to lymphoid organs may maintain enough activated T cells to control the infection efficiently but not enough to clear the peripheral foci of organisms.^{2,24,61,62}

Between the two extremes shown in Figures 2A and 2B, an unrelenting immune response to the persisting microorganism expressed in many host cells can result in serious immunopathologic conditions (e.g., chronic active hepatitis) (Fig. 2C).

In contrast to these examples of variable immune reactivity, other viruses do not initiate an immune response for a very long time, because they remain outside lymphoid tissues. For example, during a variably long period after rabies infection, the virus is sequestered in neuronal axons; similarly, human papillomavirus is found in warts (Fig. 2D).

Allografts

Unlike infection, transplantation usually results in a double immune reaction: host-versus-graft and graft-versus-host. These responses must persist long enough to allow the induction of mutual nonreactivity such as that which occurs spontaneously in dizygotic calf²⁶ and tamarin⁶³ twins when there is fusion of the two placentas, in parabiosis experiments, and after organ transplantation in some mice, rats, and pigs.⁴³

In humans, an umbrella of initially potent immunosuppression is required after either organ or bone marrow transplantation to avoid acute destruction of the leukocytes in the transplant by host cells. Failure of such treatment can be defined as the inability to control one or both of the two reactions.^{4,5,43} There are many possible clinicopathologic consequences of the mutual immunocyte engagement (Fig. 3), which are dictated largely by the condition of the recipient's immune system at the time of transplantation and by the dose and lineages of the donor leukocytes.²²

Organ Transplants

The objective of immunosuppression in the recipient of an organ transplant is control of the host-versus-graft reaction (rejection) (Table 1). However, a graft-versus-host reaction, which is usually occult, may become apparent or even lethal, particularly after transplantation of leukocyte-rich allografts such as the liver or intestine. Graft-versus-host disease is diagnosed according to conventional criteria (e.g., apoptosis in an area of dermatitis) in approximately 5 percent of patients who receive hepatic allografts. The complication causes death, often with involvement of visceral organs or bone marrow, in about 10 percent of affected patients, despite increased immunosuppression or other attempts at immune modulation.⁵

As with an overwhelming noncytopathic infection, an initially strong immune reaction that is pharmacologically suppressed may eventually be deleted by clonal exhaustion or at least reduced to an acceptable level (Fig. 2A and 3). In clinical practice, the

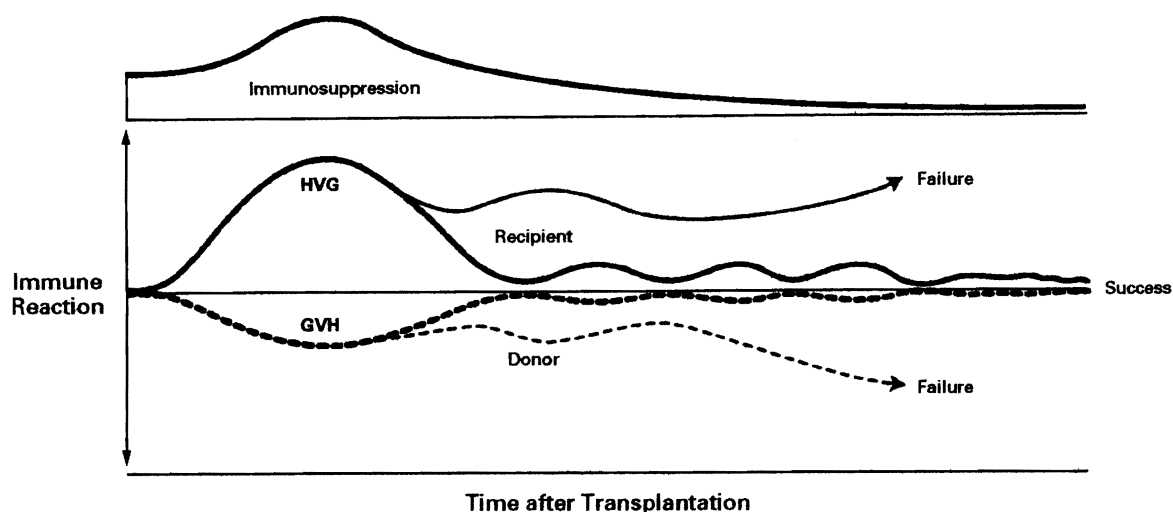


Figure 3. Contemporaneous Host-versus-Graft (HVG) and Graft-versus-Host (GVH) Reactions after Transplantation. Failure is defined as the inability to control one or sometimes both of the reactions. Acute reciprocal clonal exhaustion after successful transplantation is subsequently maintained by chimerism-dependent low-grade stimulation of both leukocyte populations, which may wax and wane.

rise and subsequent decline of the immune responses are reflected by biochemical indicators of organ function (e.g., high serum creatinine and bilirubin concentrations in kidney and liver recipients, respectively).^{29,44}

Even if the host-versus-graft reaction is not severe enough to perturb allograft function seriously, serial biopsies frequently reveal subclinical rejection that may wax and wane. In spontaneously tolerant animals, such self-resolving histopathological findings of rejection are characteristic.^{18,22,44} Although this pattern of immunologic confrontation and resolution is the same with all organs, liver allografts are more likely to induce tolerance than other organs, because they contain many hematopoietic cells that are capable of traveling to organized lymphoid tissue of the recipient, where they induce and exhaust host

anti-graft T cells and where the anti-host T cells are deleted.

If the reciprocal deletion does not occur or cannot be perpetuated despite continuous immunosuppression, the result can be acute-to-chronic rejection or, uncommonly, acute-to-chronic graft-versus-host disease (Fig. 2B). We have suggested that allogeneic leukocytes in nonlymphoid sites may leak to lymphoid organs and maintain clonal exhaustion, paralleling the "balanced" state achieved by infectious agents.^{1-3,62} In transplant recipients, T-cell responses that are inefficiently induced or of low strength would not be capable of rejecting either the chimeric cells or the organ grafts. It is also possible, however, that the resulting chronic immune response may lead to chronic rejection or even chronic graft-versus-host disease. After liver transplantation, either of these complications may resemble chronic active hepatitis (Fig. 2C).⁶⁴

It is clear that in addition to causing the host-versus-graft and graft-versus-host reactions shown in Figures 2A, 2B, 2C, and 3, the emigration of the passenger leukocytes from a transplanted organ results in a fortuitous decline in the allograft's immunogenicity,^{3-5,43} similar to that which occurs in experiments in animals when these cells are removed before organs are transplanted.^{45,46,48,49,55,56} Thus, for completely successful organ engraftment, four interrelated changes must take place in a close temporal relation: clonal deletion of the recipient's immune response, reciprocal deletion of the donor-leukocyte response, maintenance of clonal exhaustion, and a reduction in

TABLE 1. DIFFERENCES BETWEEN CLINICAL ORGAN TRANSPLANTATION AND BONE MARROW TRANSPLANTATION.

FEATURE	ORGAN TRANSPLANTATION	BONE MARROW TRANSPLANTATION
Host cytoablation	No	Yes*
HLA matching	Not essential	Critical
Principal complication	Rejection	Graft-versus-host disease
Immunosuppression-free	Uncommon	Common
Term for success	Acceptance	Tolerance

*This therapeutic step allows a relatively unopposed graft-versus-host reaction and accounts for the other differences.

the immunogenicity of the donor-leukocyte-depleted organ. In time, a stable allograft resulting from this process may come to resemble a wart^{3,43} that neither readily induces an immune response nor is readily reached by immune effector mechanisms (Fig. 2D).

Bone Marrow Transplants

Pretransplantation cytoablation renders the recipient susceptible to immune attack by donor immune cells (the graft-versus-host reaction), control of which frequently becomes the principal objective of immunosuppression (Table 1). Since complete destruction of host leukocytes is not possible,⁶⁵ the remaining cells will stimulate a response by mature or maturing donor T cells. Nevertheless, during immunosuppressive treatment, a weak host-versus-graft reaction mounted by these few recipient cells and a parallel graft-versus-host reaction mounted by the donor bone marrow cells may eventually result in reciprocal tolerance by deletion. These processes represent a mirror image of the events after organ transplantation (Fig. 2D).

OTHER MECHANISMS OF TOLERANCE

Counterregulatory mechanisms of tolerance (e.g., suppressor or veto cells and changes in the cytokine profile) may be important in some circumstances, differing according to the model and the time after exposure to antigen. However, they are neither confirmed nor understood, and they are not essential for the basic argument presented in this review about the seminal role of antigen migration and localization.

The importance of microchimerism has been questioned because of the inconsistency with which donor leukocytes can be found in blood or tissue samples from organ recipients, the development of acute or chronic rejection despite chimerism, and the inability to use microchimerism to guide post-transplantation drug weaning. These observations⁶⁶ can be readily fitted into the concept of the various balanced states that may vary with time and according to antigen migration and localization. Within this view, donor-leukocyte chimerism is a prerequisite for, but not synonymous with and not a consequence of, the evolution of organ-allograft acceptance under clinically relevant circumstances.^{4,5,43} This concept has recently been confirmed in a skin-transplantation model in mice.⁵³

ADDITIONAL IMPLICATIONS

The concepts described in this article may help us understand other as yet unexplained observations. For example, the time needed to establish transplant tolerance differs in different species (days or weeks in rodents, months or years in humans). The variability seems to correlate roughly with differences in host size, duration of gestation, and life span but not with differences in immune cells, which are structurally and functionally similar *in vitro* in all mammals.

T-cell immune function begins in the very early stages of fetal development,^{8,30,38} suggesting that antigen migration and localization is the basis for the ontogeny of the usual nonreactivity to self antigens in the same way as it is for acquired tolerance. Autoimmune disease would then reflect unacceptable postnatal perturbations of the prenatally established localization of self antigens in nonlymphoid as opposed to lymphoid tissues.⁶⁷

It will be important to determine what role the well-known leakage of semi-allogeneic fetal leukocytes into the maternal circulation has in the survival of the conceptus during pregnancy, its rejection at parturition, and the health of the mother subsequently. In a situation that is analogous to the findings after transplantation, cells of unquestionably fetal origin have been detected in the blood of women many years after childbearing, with possible associations with autoimmune disease.^{68,69}

CONCLUSIONS

Short-term effective immune reactivity, or the failure to induce an immune response at one extreme as compared with an exhaustive induction of all relevant T cells causing deletion at the other extreme, is influenced by dose, timing, route, and localization of antigen. These factors determine when, where, for how long, and with what clinical sequelae immune effector functions are or are not induced and maintained. Although the relation between infection and transplantation immunity is complicated by the presence of contemporaneous host-versus-graft and graft-versus-host immune reactions and the additional factor of therapeutic immunosuppression after transplantation, the mechanisms and rules are basically the same. The simple antigen migration-localization principle should further our understanding of the events that occur with or without therapeutic intervention in a variety of infectious, neoplastic, and autoimmune diseases or after transplantation, and it may offer improved rationales for prevention and treatment.

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