The Biology of Acute Transplant Rejection

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An intriguing and increasingly understood facet of immune responses is the ability of a recipient to destroy a foreign tissue or organ graft. The phenomenon of acute rejection of an allograft involves a series of complex and inter-related cellular and humoral events, culminating in graft death. Some of the current thinking surrounding this phenomenon is reviewed.

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THE IMMUNE SYSTEM has evolved from primitive beginnings in lower organisms to one of remarkable diversity and sophistication in mammals. Its function is to protect the host throughout its lifetime by inactivating or destroying foreign antigens, presumably including mutant host cells bearing potentially malignant characteristics. Because the realization that this critical aspect of the host defense mechanisms is both monitored and mediated primarily by lymphoid cells, increasing interest among biologists has become centered on lymphocytes, their behavior and function, their interactions, and their products. These cells are ubiquitous throughout the body, make up approximately 20% of circulating leukocytes, and are concentrated in thoracic duct lymph, bone marrow, submucosa of the gut, spleen, lymph nodes, thymus, tonsils, and Peyer's patches. Overall the lymphoid tissues comprise an organ of considerable size.

Because the phenomenon of rejection has become recognized as a lymphocyte-directed immunologic event, investigations into this dramatic, complex, and powerful panoply of host defenses brought into play by the stimulus of an allograft and leading to its destruction have increased dramatically during the past few decades. Indeed definition of these responses, sharpened by coincident advances in several related biosciences, which include immunogenetics, cell biology, molecular biology and pharmacology, have led to an explosion in knowledge undreamed of by the original pioneers in these fields.

The modern era of transplantation biology was opened by Medawar during World War II with his observations with Gibson on the differences in behavior between skin autografts and allografts placed on severely burned patients. In subsequent experimental studies in rabbits, he described a progressive infiltration of the graft and graft bed by host mononuclear cells leading to its destruction about 1 week after placement.[1] He also found that when additional skin from the same donor was later transplanted to these animals, rejection of the 'second set' grafts occurred more rapidly than the initial event. The immunologic nature of these phenomenon were reinforced by dramatic changes in the lymph nodes draining orthotopic skin allografts, which included the appearance of large numbers of lymphoid cells in cortical nodules and marked proliferation of plasma cells in medullary cords.[2] The coincident development of genetically defined inbred mouse strains by Snell and others[3] provided the opportunity to use reproducible animal models in the burgeoning biology of transplantation immunology. Using such inbred strains, Mitchison[4] and Billingham, Brent, and Medawar[5] conferred immune reactivity to otherwise naive mice by adoptive transfer of lymphocytes from animals of the same strain that had previously responded to a particular tumor or skin graft; as such immunity could not be transferred consistently with serum, antibody responses were considered of secondary importance in the acute destruction of foreign tissues. Gowans[6,7] then showed that many small lymphocytes recirculated continuously in the body and were 'immunologically competent.' Subsequently histocompatibility antigens, defined at cellular and molecular levels, were found both to stimulate and provide targets for host immune responsiveness. More recently, rapid advancements in hybridoma technology with increasing availability of monoclonal antibodies (MAb) directed specifically against individual antigenic determinants are allowing more complete char-

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acterization of the cellular cascade mediating rejection, as well as progressive understanding of the actions, interrelationships, influences and contributions of cell populations, subpopulations, and their factors in this extraordinary host event.

Based on the experience in clinical transplantation during the last three decades, patterns of the host immune responses against foreign organs or tissues have become appreciated, each with its relatively discreet histopathology, host mechanisms of destruction, timing, and prognosis. Hyperacute rejection is considered primarily a humoral event manifested by rapid interaction between cytotoxic antibodies circulating in the presensitized recipient and graft antigen expressed predominantly on its vascular endothelium. Inflammatory mediators including those of the complement, kinin, and coagulation systems are activated and released, resulting in vascular dissolution, interstitial hemorrhage, microvascular thrombosis, and graft death. Accelerated rejection includes both humoral and cellular components of host immunity and occurs within a few days of engraftment in recipients previously sensitized against donor antigens. Acute rejection is primarily a cellular (T lymphocyte mediated) phenomenon that appears 1 week or later in desensitized hosts. Chronic rejection implies gradual fibrosis of the graft that is manifested by declining function over months or years, and is thought to be predominantly on a humoral basis. As most is known currently about its biology, this review will be limited to the events of acute rejection, although it should be stressed that the above conditions are not discrete entities unto themselves but are undoubtedly part of a spectrum or continuum of host immune activity.

The Cells Responsible for Acute Rejection

Progressive infiltration of the graft substance by host mononuclear cells is characteristic of acute rejection of allogeneic tissues of all types. Within a few hours of revascularization, a few small host lymphocytes infiltrate perivascular areas and then scatter throughout the graft substance. As the inflammatory reaction proceeds, macrophages became more obvious, with disruption of pericapillary tissues, progressive interstitial inflammation, and eventual tissue necrosis. At the same time, numbers of lymphocytic cells, both mature and blastic, increase expedientially in recipient lymphoid compartments.

The host cells entering rejecting allografts and migrating to lymphoid tissues include T and B lymphocytes, macrophages, and natural killer (NK) cells.[1,8,9] The primary responsibility of T cells in the acute destructive process has been emphasized *in vitro* in the lymphocyte-mediated cytoxicity assay by their ability to lyse donor target cells by direct interaction without the need for antibody,[10] and *in vivo* by the inability of T-cell–depleted animals to reject allografted tissues.[11] The increasing availability of specific MAbs also has allowed fractionation of T lymphocytes into their subpopulations, cytotoxic/suppressor (Tc/s) cells, and T helper/inducer (Th) phenotype.[12] The current phenotypic nomenclature for Tc/s and Th lymphocytes is CD8 and CD4, respectively; CD8 is comparable to OKT8 or LEU-2, LYT-2 and OX8 in human, mouse and rat, respectively, while CD4 is comparable to OKT4 or LEU-3, or L3T4, and W3/25, respectively. It should be emphasized, however, that identification of surface antigens expressed on resting Tc/s and Th cells wrongly suggests a direct correlation between phenotype and function; indeed particular states of host immune responsiveness may change cell relationships and alter phenotype. Thus it has become apparent that the use of MAbs directed against activation markers such as specific receptors developing on the surfaces of antigen-activated cells has allowed better understanding of function of various cell subpopulations than their identification by phenotype alone.[13] The sharing of a phenotype by Tc/s also presents a conceptual problem because these populations or a single Tc/s population may either destroy allogeneic cells or suppress their alloaggressive functions, behavior that is presumably dependent on particular conditions of host activation.

In addition to T lymphocytes, other cell populations infiltrate rejecting grafts. B lymphocytes, when allostimulated, differentiate into antibody-producing plasma cells; large numbers of immunoblasts and plasma cells are seen in host spleen within a few days of engraftment. B cells from the graft infiltrate secrete both nonspecific antibodies and those specific against the donor.[14] However, despite their obvious activity, their actual role in acute cell-mediated rejection remains enigmatic and not well defined. Natural killer cells, a population of cytotoxic, non-T,non-B cells, destroy certain tumor cells *in vitro*; although they infiltrate organ allografts rapidly, deletion experiments in graft recipients using specific anti-NK cell antibodies show that they are not critical in the acute rejection process.[15] In antigen–antibody interactions, however, where the constant Fc portion of the antibody is exposed, NK cells adhere to the Fc molecules and presumably contribute to the rejection process.

Macrophages act both as antigen-presenting cells that initiate immune responsiveness and as alloaggressive cells that contribute to graft destruction.[16] Their unrelated roles in the host defenses are several. (1) Macrophages may carry or process antigen activated lymphocyte populations by direct contact. (2) They may act as the principle site of control by immune response genes. (3) Receptors on their plasma membranes may cause destruction of antibody-coated target cells, activation of B cells by antigen, and location of antigen–antibody complexes. (4) They may elaborate cytokines when activated, particularly in-

terleukin-1 (IL-1), which in turn activates CD4+ lymphocytes. Lymphokines released by activated CD4+ cells, particularly gamma interferon (IFNγ), may stimulate naive macrophages to become alloaggressive against the graft.

Mechanisms of Acute Allograft Rejection

Like a neurologic arc, the immunologic host responses to foreign tissues have been conceptualized as comprising two limbs, an afferent or sensitizing limb and an efferent or effector limb.[7] Following interactions between circulating host lymphocytes and graft antigen or antigen-presenting cells, specifically sensitized effector cells enter the graft substance *via* its vasculature and presumably cause or at least trigger its destruction by attracting large numbers of nonspecific lymphocytes and macrophages to its substance. Other sensitized cells proliferate in host lymphoid tissues. The actual biologic mechanisms by which these events occur are gradually becoming unravelled.

Graft Immunogenicity

Although not well defined, cellular elements within the transplanted tissues themselves may possess antigenic determinants of varying immunostimulatory capacities. Epidermal cells, vascular endothelium, donor leukocytes, or dendritic cells (DC) isolated from the graft substance, spleen, or afferent lymph may cause histoincompatible allogeneic leukocytes to proliferate vigorously *in vitro*; *in vivo* transfer of such cells can elicit antibody production by the host or induce rejection of established organ allografts following transfer.[17,18] 'Passenger leukocytes' residing within the graft have been thought important in host sensitization. Removal of DCs from mouse pancreatic islets before transplantation, for instance, renders the islets nonimmunogenic, while administration of DCs before engraftment may cause rejection of tissues transplanted thereafter.[19] Such findings are not uniform, however, because heart grafts are rejected in an accelerated fashion in some mouse strains that had received DCs before transplantation but not in those that were given previous blood transfusions.[20]

There may be several subpopulations of DCs. Langerhans cells in skin are derived from bone marrow. Their reduction by donor pretreatment with x radiation, corticosteroids, or other leukocytic agents increase skin graft survival.[17] These cells migrate from blood to spleen, enter T-cell–dependent areas from the splenic marginal zone, and interact closely with T-cell subpopulations, primarily Th.[21] Not only can they present antigen to sensitized T cells but they can activate resting T cells. Lymphoid DCs, isolated from spleen, are immunostimulatory to T cells and have characteristic phenotype but lack markers for macrophages and lymphocytes. They are widely scattered throughout many tissues in the body, both lymphoid and nonlymphoid, and migrate in blood and lymph. In addition DCs from grafted tissues may migrate to spleen,[22] an observation at variance with the usual dogma that suggests that sensitization of host cells occurs within the graft itself and not in host lymphoid tissues. Migration of DCs from tissue to tissue *via* the blood stream and lymph seems to be a physiologic process that may amplify sensitization of the host to an antigen.

Tissue-specific antigens also may be important components of immunogenicity; transplants of pure epidermal cells from skin bearing putative 'skin specific antigens' are rejected acutely, while different antigen systems in organ allografts may initiate their rejection.

The immunogenic variation between skin and primarily vascularized allografts also has been explained on the basis of the route of host sensitization. Unlike organ grafts, which are revascularized promptly, skin grafts are laid directly on the recipient dermal bed. Complete revascularization apparently is not necessary for immunostimulation because a 3- to 4-day exposure to a first-set skin graft will sensitize the host; the more rapid re-establishment of lymphatic drainage within that period allows percolation of particulate graft antigen from skin to regional lymph nodes, a more important route of sensitization in this context than migration of passenger leukocytes to recipient lymphoid tissues or host lymphocytes circulating to the graft.[23] The importance of lymphatics in host sensitization in particular anatomic locations has been emphasized by the relative protection conferred on tissue allografts placed in 'immunologically privileged' sites, which include the anterior chamber of the eye, brain, hamster cheek pouch, and vascularized skin pedicles with surgically interrupted lymphatics.[24]

In contrast direct revascularization of organ allografts is critical for host immunization; the tempo of rejection is unaffected by isolation of kidney grafts from recipient lymphatics by placing the organ in a plastic bag.[25] Nor is the rejection rate influenced when efferent lymph from the graft is diverted.[26] Circulating host effector cells become sensitized by contact with the antigens of a primarily revascularized graft. Many cells may migrate into the organ; those specifically sensitized to graft antigen may be retained selectively within it and in turn may attract and mobilize large numbers of uncommitted potentially alloaggressive cells by direct interaction. That only a small population of specifically sensitized cells is necessary to trigger the effector responses has been shown by radiolabeling experiments tracing lymphocytes sensitized against different antigens; there is always selective infiltration of small but critical numbers of antigen-specific cells into the appropriate allograft.[27]

Antigen Presentation and Recognition

Antigens on allogeneic cell surfaces allow the host to recognize that the transplanted tissue is not 'self.' It has been long known that the immune responses between genetically dissimilar humans are directed against a single cluster of alloantigens, designated human leukocyte antigen (HLA) and encoded by major histocompatibility complex (MHC) genes found on chromosome six.[28] There are two MHC antigen groups: class I (HLA-A and B) and class II (HLA-D and DR), both prime targets for host immunoreactivity. These are expressed characteristically on various mammalian tissues, may be up or down regulated by host cells and their products, and may serve to trigger graft rejection differentially. Class I antigens are relatively ubiquitous and are constituitively expressed throughout somatic cells; these interact exclusively with and activate CD8+ T lymphocytes. Class II antigens are distributed more selectively throughout lymphoid tissues, on DCs, Langerhans cells in skin, circulating B lymphocytes, and monocytes. Their distribution on vascular endothelial cells is a particularly important site for immune injury because endothelium is exposed continuously to circulating effector cells and their products. CD4+ T lymphocytes are activated selectively by class II antigens. In addition cell mediators or lymphokines can regulate expression of MHC antigens selectively by inducing different class II gene products or promoting class I antigens differentially over class II. Interferon γ, for instance, can upregulate class II antigen on several cell types, including lymphocytes, pancreatic beta cells, vascular endothelium, and renal tubular cells; this upregulation increases antigen presentation and amplifies graft immunogenicity.[29] Transplanted tissues or their components also may differ in expression of these antigen; in the rat, class I expression develops more rapidly in the allografted kidney than in the heart, increasing many times (40x) within a few days of transplantation. In contrast the kinetics of class II induction is similar in kidney and heart grafts.[30]

Recognition of graft 'foreigness' by T lymphocytes requires both the MHC molecule and the alloantigen.[31] The spatial configuration of this event involves interaction between the antigen receptor of specific T cells and a foreign peptide bound to a groove in the MHC molecule; this complex arrangement allows presentation of alloantigen to the T-cell receptor *via* its alpha and beta cell-surface molecules.[32] The immunologic function of the remainder of the T-cell antigen receptor (gamma, delta, and zeta chains) is not clear. Genetic rearrangements of alpha and beta genes, covalently linked by disulfide bonds, produce combining sites on the cell surface that can recognize virtually any antigen. The phenotypic structures CD4 and CD8 also act as accessory molecules to increase the avidity of the interaction between the MHC antigen and the T-cell receptor. Once specific alloantigens have been recognized, the T lymphocyte becomes activated, a process involving complex intracellular changes that result in clonal expansion, with differentiation and proliferation of a new generation of antigen-specific T cells. Antigen activation of CD4+ Th cells causes production and release of cell products and expression of various surface receptors. At the same time, activated CD8+ cells develop receptors for interleukin-2 (IL-2) and IL-2 receptor (IL-2R).

Antigen recognition by T lymphocytes alone is not thought to be sufficient to trigger host events. A two-signal process of lymphocyte activation has been suggested, involving binding of transplantation antigens to T-cell surface receptors (signal 1), which coincidentally receive a costimulating signal 2 from antigen-presenting cells.[33] Activation and differentiation of CD8+ T cells occurs after both signals but not by interaction with serologically defined antigens on the surfaces of allogeneic cells. CD8+ precursors, activated by class I antigens, differentiate and proliferate further after interaction with IL-2 produced by CD4+ lymphocytes, themselves activated both by class II antigens and IL-1.[8,34]

Responses in Host Lymphoid Tissue

The most marked histologic changes occurring after placement of orthotopic skin allografts take place in regional draining lymph nodes following the rather prompt (about 3 days) reconstitution of lymphatic drainage. In contrast, in recipients of vascularized organ grafts, the spleen responds most dramatically.[35] Within 2 days of engraftment, large pyroninophilic immunoblasts proliferate in the peripheral periarterial lymphocyte sheaths then migrate into the red pulp where they differentiate into plasma cells. These antibody-producing cells, which triple the volume of the red pulp by the time of actual graft rejection, gradually decrease in number thereafter and are replaced by immunoperoxidase-negative large and small lymphocytes, changes associated with a rapid antibody response occurring 3 to 5 days after transplantation and peaking by 7 days.

Lymphocyte migration studies have emphasized the importance of massive shifts in cell populations to host lymphoid and nonlymphoid tissues following the antigenic stimulus of an organ graft. Under physiologic conditions T lymphocytes recirculate continuously between blood, lymphoid tissues, and lymph.[6] In the event of an antigenic stimulus in the gut, from a site of inflammation in skin such as a delayed-type hypersensitivity (DTH) reaction, or from placement of an allograft, such migration patterns change drastically to allow the antigenic message to disseminate as widely and promptly as possible

throughout the host immune system.[36] The spleen is an important point of interaction between host lymphocytes and graft antigen brought by antigen-presenting cells, or with lymphokines released into the circulation by antigen-activated cells. It also acts as a site for antigen recognition and activation by naive host cells. Under physiologic conditions normal splenocytes localize relatively selectively in spleen and away from mesenteric and peripheral lymph nodes; during acute rejection, however, marked homing of sensitized cells occur in lymph nodes and Peyers patches of the gut, presumably to disperse the antigenic message more generally throughout host lymphoid tissues. In contrast the lymphoid cells that accumulate in spleen during the early phases of the acute rejection process diminish rapidly thereafter because of apparent migration to the graft site.

At least some of the rapid changes in lymphocyte migration patterns after an antigenic stimulus can be explained by the development of adhesion molecules both on antigenic cell surfaces and on lymphocyte membranes.[37] As noted CD4 and CD8 molecules increase the avidity of the interaction between the MHC graft antigen and the T-cell receptor. In addition other adhesion molecules, distinct from antigen-specific receptors, may influence homing patterns of lymphocytes.[38–40] Leukocyte function-associated antigen (LFA-1) causes lymphocytes to bind to high endothelial venules in lymph nodes; once there they migrate between the endothelial cells and enter the nodal substance, where they pursue their immunologic function. Other adhesion molecules on endothelial surfaces, endothelial-leukocyte adhesion molecules or intracellular adhesion molecules, for instance, may alter physiologic migration patterns of recirculating lymphocytes and increase the immunologic message to the entire host. One can visualize how a single antigenic site can engender systemic immune responsiveness in an effective and rapid fashion.

The Efferent Limb

Understanding of the actual means by which sensitized host cells actually destroy a graft remains elusive. The current consensus on these events have evolved through study of serial histologic changes within the rejecting graft, identification by phenotype and assessment of function of cell populations and subpopulations entering the graft from the circulation, and appreciation of the role of humoral destructive factors.[8,41,42] In addition, once the rejection events are well underway, regulatory host mechanisms are initiated whereby these protean and powerful alloaggressive responses are tempered, attenuated and reversed to restore the host to immunologic homeostasis.

The Cells Involved

Acute rejection is primarily a T-lymphocyte–mediated phenomenon; the survival of chicken feathers transplanted to athymic T-cell–deprived nude mice is a compelling affirmation of this observation.[43] Until recently it was assumed that the CD8+ cytotoxic T lymphocyte was the critical effector element in the process because of its ability to lyse donor target cells *in vitro*.[10] However with the appreciation that CD4+ and CD8+ T cells could be stimulated differentially by class II and class I MHC molecules, respectively, has come the realization that CD4+ cells may activate other lymphocyte populations, primarily through elaboration of IL-2.[44] Although the seminal role of these cells in DTH responses in activating and mobilizing macrophages to the site of inflammation is well recognized,[34] both CD4+ and CD8+ T-lymphocyte subpopulations are necessary to produce rejection in transplantation models (Fig. 1). Such interaction has been shown most obviously by reconstitution experiments in immunodeficient hosts; T-cell subpopulations, alone or in combination and with or without the addition of IL-2, were adoptively transferred into T-cell–depleted animals that were otherwise unable to reject their organ allografts.[11,45] Acute rejection could only be recreated by reconstitution with all T-lymphocyte elements and IL-2.

The nature of the graft, the degree of host sensitization, and histocompatibility differences between donor and recipient also may influence the relative contribution of the T-cell subsets. Current thinking emphasizes CD4+ T helper cells to be the primary, initiating, and organizing component of immune responsiveness against allografted tissues, with the Tc/s CD8+ subpopulation recruited secondarily to the site to complete the acute rejection process.[8,41,42,44] In addition to the activity of alloaggressive macrophages, the cytotoxic effects of this latter subpopulation presumably destroy allogeneic tissue directly. Following rejection both T-cell subsets may then revert to a resting state as memory cells. In contrast both CD4+ and CD8+ subpopulations rapidly mediate the accelerated rejection of a second donor strain transplant.[46] Overall it appears that graft rejection reflects different contributions of not one but multiple effector mechanisms.

Cell Products and Their Receptors

With definition of the cell populations involved in the immunologic host responses against allografted tissues has come appreciation of the critical importance of their products, the cytokines and lymphokines (Fig. 2). Various effector cell populations may produce one or more factors that, in turn, activate (or suppress) the function of other cell subpopulations. As noted antigen-activated macrophages elaborate IL-1, a monokine that stimulates CD4+

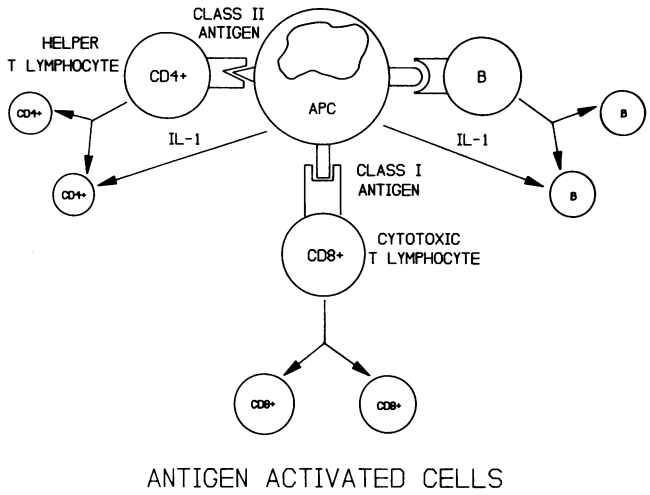

FIG. 1. Various lymphocyte populations are activated directly by graft antigen or by antigen-processing cells (APC) expressing MHC antigens. IL-1, a cytokine elaborated by APCs or activated macrophages, in turn activates CD4+ lymphocytes and B cells.

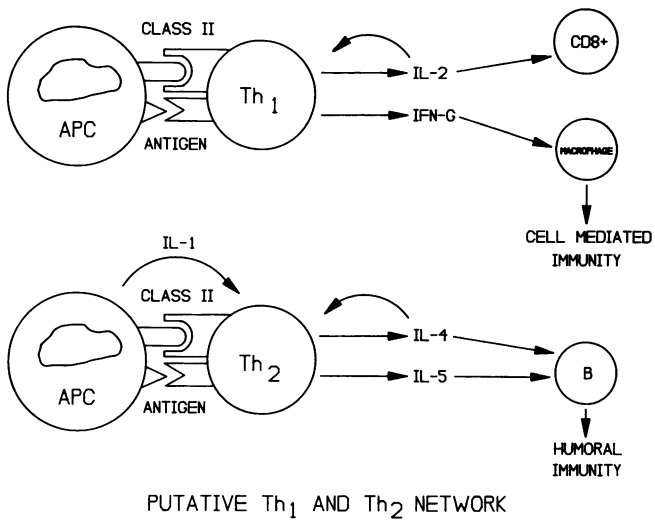

FIG. 3. Two putative Th subpopulations are thought to elaborate lymphokines differentially.

cells to give off a series of humoral mediators. Other cytokines released by macrophages also may be important in graft destruction and include tumor necrosis factors beta (TNFβ; cachectin) and alpha (TNFα; lymphocytotoxin).[47] In addition TNF released locally by cells infiltrating the graft may (with IFNγ) stimulate further MHC antigen expression by the graft.

Activated CD4+ T cells are critical in the early phases of the rejection cascade because of their ability to elaborate several lymphokines. Some researchers have thought that the CD4+ Th lymphocyte population can itself be divided into at least two subclasses (Th_1 and Th_2), each of which elaborate distinct lymphokines with their own properties and actions (Fig. 3).[48] The most important, Th1-derived IL-2, stimulates activated T and B lymphocytes to differentiate and proliferate. Other interleukins (there are now eight recognized) stimulate B-cell maturation or effect cell populations in the bone marrow. Th1-derived IFNγ has several putative roles in immunoresponsiveness.[49] It amplifies the entire process by inducing and intensifying class I and class II MHC antigen expression on the graft, stimulates B cells to increase antibody production, and augments alloaggressiveness of previously uncommitted macrophages and monocytes. In addition it may increase adhesiveness of lymphocytes to an antigenic site by enhancing expression of LFA-1 on their surfaces.[40] In some systems IFNγ antagonizes activity of IL-1 and IL-4; however transfer of cloned material to an unresponsive host may produce acute rejection of otherwise well-functioning organ grafts.[50]

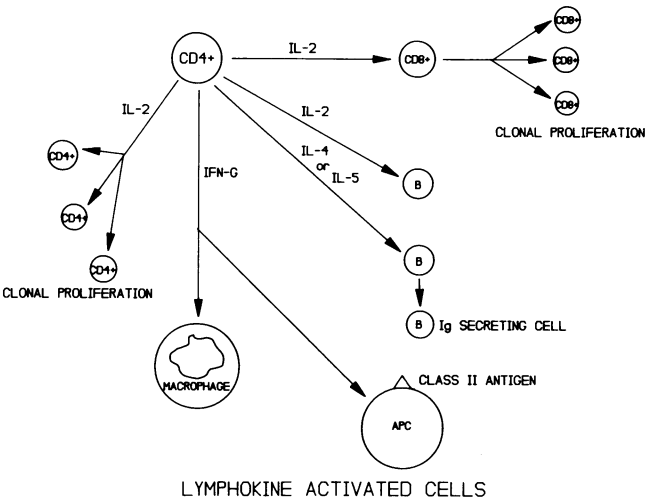

FIG. 2. Activated CD4+ lymphocytes produce several lymphokines, including gamma interferon (IFN-G), which stimulate other cell populations.

Early stimulation of T lymphocytes by antigen causes them to develop receptors on their surfaces for transferrin, insulin, IL-1, IL-2, and presumably other products as well.[51] The development of high-affinity surface receptors for IL-2 on most activated CD4+ and CD8+ T lymphocytes, some B cells, DCs, and macrophages has been thought particularly important in the rejection cascade. Binding of this lymphokine to its receptor is followed by internalization of the entire complex, which transduces the signal for proliferation and clonal expansion of the activated cell population and drives the entire rejection event forward. This antigen-activated cell population is relatively small; about 15% of infiltrating cells in rejecting rat cardiac grafts are IL-2R positive and more than 20%

of lymphocytes express this receptor in a popliteal lymph node model.[13,52]

The importance of activated IL-2R–positive cells in rejection has been shown in several species, including humans, by specific anti-IL-2R MAbs.[53] Using these antibodies it has become clear that IL-2R are heterogeneous in terms of binding and structural characteristics; low, intermediate, and high affinity forms have been described. Interleukin-2R–targeted therapy prolongs skin and organ graft survival dramatically in some mouse strains.[54] In an extensive series of experiments in rat allograft models, the role of epitope defined on target cells was found to be critical; that is some antibodies directed against particular epitopes of the receptor complex prevent or reverse rejection; others directed against functionally different epitopes, although active *in vitro*, are therapeutically ineffectual. Monoclonal antibodies against different IL-2R epitopes also were found to be additive or even synergistic, suggesting more complete coverage of the three-dimensional molecular receptor structure.[53] In addition the effectiveness of antibody isotype may vary. At least in rats, the alloaggressiveness of IL-2R–positive cells is decreased by actual lysis, not merely by covering the receptor molecule.[55] As a result IL-2R–positive cells from the graft infiltrate were virtually eliminated by these antibodies, with significant increase in graft survival. In contrast cells with suppressor activity (Ts) were spared. Some anti–IL-2R MAbs act synergistically with subclinical doses of cyclosporine, an observation of potential clinical importance.[56] The potency of these MAbs in increasing graft survival in subhuman primates and in humans, although less dramatic than in smaller laboratory animals, also have been interesting enough to stimulate clinical trials.[57] At the very least, early use of such antibodies in human renal transplant recipients delay the first rejection event.

Regulation of Immune Responsiveness

After the rejection episode is completed and the graft has been destroyed, the host responses must return to baseline; thus intrinsic control mechanisms must arise to reverse the immune processes. As graft antigen expression progressively diminishes, clonal expansion of lymphocyte subpopulations slows with eventual reversion of the cells to their resting states; lymphokine transcription, messenger RNA-encoding IL-2R, and lymphokine production gradually cease. In addition suppressor mechanisms may be brought into play to reverse the inflammatory process.

The concept of suppression as an active immunoregulatory mechanism is intrinsic to the study of transplantation biology. It originated in studies of tolerance whereby fetal or neonatal animals, initially exposed to specific antigens, became permanently nonreactive to the same antigens when they were presented later in life.[58] Tolerant states then were found to be thymus dependent; specific immunologic unresponsiveness could be induced in the normal host by transfer of thymocytes from tolerant animals, while removal of such tolerance-inducing cells in bulk, as by thymectomy, may cause abrupt allograft rejection in several experimental models.[59,60] It has shown subsequently that various immunosuppressive modalities, both chemical and biologic, can inhibit the effector responses of graft recipients but spare cell populations with suppressor capabilities that contribute to the development of specific host unresponsiveness.[61]

T lymphocytes with suppressor characteristics presumably are critical in producing and maintaining immunologic homeostasis in a host that is exposed throughout its life to multiple antigenic stimuli. Although there is ongoing debate about their existence by basic immunologists, Ts (or at least cells with suppressor function) have been identified both *in vitro* and *in vivo* in various immunologic models and disease states. In transplantation models in which allografts survive for prolonged periods in unresponsive recipients, Ts often are demonstrable in the maintenance phase of unresponsiveness; indeed a common denominator of long-term host unresponsiveness seems to be the activity of such cells or their products. These experimental systems include neonatally induced tolerance, allograft recipients undergoing total lymphoid x radiation, or enhanced animals pretreated with antigen and/or antibody.[62] They also include engrafted animals conditioned with donor blood and anti-lymphocyte serum and those immunosuppressed with cyclosporine.[56,61]

It is interesting that in several models the CD4+ and CD8+ phenotypes shift between the early and later phases of unresponsiveness.[63] Because of our inability to differentiate between effector and suppressor cells by phenotype, it is possible that suppressor function can supersede effector activity in cells of the same phenotype; indeed this same cell may be responsible for both processes, depending on the circumstance of host immune stimulation. Thus Tc/s may not be only responsible for completing graft destruction but for halting the systemic effects of immunity. Alternatively more than one population of Ts may be responsible for inhibition of alloresponsiveness; indeed a cascade of suppression may exist that involves cell-to-cell contact and sequential activity of lymphocyte products, all orchestrated by cells differing in phenotype, MHC restriction, and allospecificity (Fig. 4).[64] Originally identified in a murine DTH model, the suppressor cell pathway has been shown to exist in transplant models by the demonstration of distinct T-cell subsets appearing during the course of prolonged rat renal allograft survival.[62] Three Ts subpopulations have been described: Ts_1 inducer/suppressor cells, Ts_2 transducer cells, and Ts_3

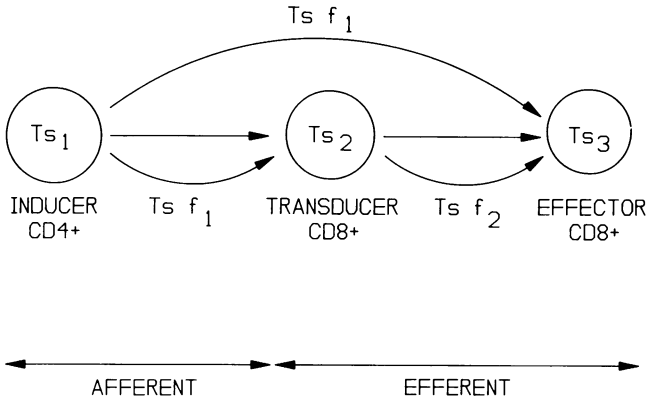

FIG. 4. The postulated suppressor cell network is shown.[64]

auxiliary or effector suppressor cells. Ts_1 and Ts_3 may bear antigen-binding receptors with idiotype-related determinants, whereas Ts_2 may have receptors for anti-idiotypes. Finally some Ts subsets may release factors that mediate both specific and nonspecific suppression by other cell subpopulations.

Thus the concept of a series of controlling or immunoregulatory mechanisms appearing as an integral part of the immune system is becoming increasingly appreciated. A balance must be maintained between effector mechanisms and suppression in acute host alloresponsiveness whereby allograft destruction ultimately is stopped by suppressor mechanisms and the animal restored to immunologic normalcy. The appearance of Ts in the later stages of rejection, regardless of phenotype, apparently represents such a host protective mechanism. Whether Ts that emerge during the rejection process represent the same populations as those governing graft acceptance in immunomodulated hosts is unknown, but seems likely. Nor is it known whether such cells are involved in the immune regulation that occurs throughout the life of an animal barraged constantly by environmental antigenic stimuli presented through skin or gut.

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