Commentary

Transplantation tolerance: Fooling mother nature

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Foreignness is threatening. The immune system, the primary arbiter of self and nonself, will reject an organ transplant unless constrained by life-long administration of multiple immune suppressive drugs. Immunosuppressive therapy, while facilitating graft success, heightens susceptibility to infection and the development of malignancy. The challenge then is to educate the immune system to tolerate the allograft in the absence of exogenous immunosuppressants. Deletion of autoreactive T cells and B cells, and immune mechanisms including suppressor and anergy, have all been advanced as the basis for tolerance to self-antigens, and the avoidance of autoimmunity (1–3). The central question is whether the principles of self-tolerance can be exploited to achieve allograft permissive tolerance, the Holy Grail of the transplantation biologist.

In this issue of Proceedings, Azuma et al. (4) inform us of a potential clinically applicable strategy for the creation of transplantation tolerance and preservation of long-term allograft function. They demonstrate that blockade of the CD28/B-7 costimulation pathway (5–8), in a rat renal allograft model, prevents the development of chronic rejection. It is shown that a single application of CTLA4 Ig, a recombinant fusion protein of the T-cell surface molecule CTLA4 and IgG1 heavy chain, reduces cellular traffic into the allografts, constrains intrarenal display of mRNA encoding T-cell and macrophage proteins and prevents histological and functional deterioration of the allograft.

The very first successful renal transplantation was performed in 1954 at the then Peter Bent Brigham Hospital (Boston) by a multidisciplinary team led by Dr. Joseph Murray. The current report, communicated by Dr. Murray and coming from the same pioneering institution, invites consideration of new knowledge of T-cell immunobiology and mechanisms of rejection that can contribute to the development of tolerogenic regimens in the clinic. Accordingly, I shall comment on the signaling features of T lymphocytes—the primary cells responsible for allograft rejection—and the mechanisms of rejection from the perspective of finer regulation of the allograft repertory.

Transplantation Antigens and T-Cell Signaling

Donor major histocompatibility complex (MHC) antigens are the primary transplantation barriers, and the clonotypically distributed T-cell receptor α, β heterodimers (TCR) are responsible for the recognition of antigenic peptide displayed by MHC antigens on antigen-presenting cells (APC) (9, 10). The recipient’s T cells might react directly with the antigen presented by the donor APC (direct recognition) or recognize donor antigen displayed by self-APC (indirect recognition). The direct and indirect pathways might have differential sensitivity to immunosuppressive drugs, and also might participate differentially in the rejection process.

This TCR-derived signal is necessary but insufficient in itself to fully activate T cells, and plenary activation is dependent upon the informative interactions among the T-cell surface costimulatory molecules and their specific counter receptors on the APC (11, 12) (Fig. 1). From a transplantation perspective, these cell surface proteins represent candidate targets for the subversion of graft-destructive immune responses. The CTLA4 Ig fusion protein, used in the current study to prevent chronic renal allograft rejection, and in additional studies to prolong the survival of islet xenografts (14) and cardiac allografts (15), and, under certain circumstances, induce transplantation tolerance (16, 17), is expected to prevent the generation of costimulatory signal(s) resulting from the interactions between CD28 antigen (highly homologous to CTLA-4 antigen) and members of the B-7 family (5–8). This lack of costimulation can heighten T-cell activation requirements since CD28 costimulation is reported to reduce the activation threshold via TCR (18).

The blockade of the CD28/B-7 pathway is an effective, but not exclusive, route for achieving transplantation tolerance. Antigen-specific tolerance and/or long-term graft function have also been realized in preclinical models by targeting other cell surface proteins, LFA-1 (lymphocyte function-associated antigen) and ICAM-1 (intercellular adhesion molecule) (19), CD40 ligand (20), or the CD2 antigen (21). A mechanistic explanation for the beneficial outcome following blockade of costimulatory signals is that whereas the delivery of both antigenic and costimulatory signals engenders graft-destructive immunity, TCR engagement in the absence of a costimulatory signal results in graft-adaptive immunity. Indeed, elegant data exist that TCR occupancy, in the absence of costimulatory signals, results in antigen-specific T-cell nonresponsiveness (Fig. 2 and reviewed in reference 22). This cellular paralysis, better characterized in vitro than in vivo, has been termed T-cell anergy. Molecular studies have shown that the p21 ras kinase, transcription factors such as AP-1 and NF-AT, and the growth promoting gene IL-2, are not fully activated and/or expressed in anergized T cells (22). It should be pointed out that alternate pathways, in addition to blockade of costimulatory signals, exist for the induction of T-cell anergy. Altered peptide ligands [analog of immunogenic peptides in which the amino acids contacting the TCR have been substituted (24)], TCR downregulation (18), the multifunctional cytokine IL-10 (25), have all been shown to induce T-cell anergy in vitro (Fig. 2). The cellular and molecular phenotypes of different T-cell anergy induction protocols share some but not all features (22).

Why should interruption of costimulatory signals result in long-lasting antigen-specific nonresponsiveness and not merely in unresponsiveness that is appreciable only at the time of first encounter with the antigen? What is responsible for this immune memory? The definitive answer is not apparent. It is easier to ascribe the first encounter non- or hyporesponsiveness to the lack of stimulating factors conducive to cell growth. To explain the persistence of a T-cell unresponsive phenotype, one needs to invoke the emergence of inhibitory factors. Indeed, inhibitors of p21 ras kinase and of IL-2 transcription have been identified in anergic T cells and advanced as mediators (22). The lack of costimulation may also have additional consequences besides T-cell anergy. In view of the observation that CD28 signaling can function as a cell survival signal (26), blockade of the CD28/B-7 pathway can result in the deletion, by apoptosis, of alloantigen reactive T cells.

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trathyritic inoculation of donor cells (31), induction of a mixed chimeric state wherein the donor bone marrow-derived cells engender antigen specific unresponsiveness (32), and exploitation of the Fas/Fas ligand pathway (33) represent approaches that are worthy of exploration in the clinical settings. The Fas/Fas ligand pathway is considered important for the immune privileged nature of certain sites and tissues (34–36).

Allograft Rejection

Acute rejection is a T-cell-dependent process and one in which multiple additional cell types including B cells, monocytes/macrophages/dendritic cells, and natural killer cells participate, and cytotoxic effector and delayed-type hypersensitivity mechanisms dominate (37–39). Although frequent in the first year of transplantation, acute rejection is usually treatable with current therapy. Molecular investigations suggest participation of cytotoxic attack molecules such as granzymes, perforin, and Fas ligand, and of immunoregulatory cytokines such as IL-2, IFN-γ and IL-10, in the acute rejection process (39–41).

Chronic rejection is a relentlessly progressive form of graft dysfunction, spanning years to decades (42–44). No effective therapy exists for this mechanistically unresolved disorder. It complicates all solid organ transplants (kidney, heart, lung, and liver), and is histologically characterized by fibrosis and vascular disease. Immunologic (antigen-dependent) and nonimmunologic (antigen-independent) pathogenic mechanisms have been invoked, and it is likely that these are complementary rather than mutually exclusive mechanisms (13, 42–44).

Is it possible to prevent the development of chronic rejection, and is it within our quiver to restrain its progression? Is there a start point and/or a common final pathway that can be targeted? The report of Azuma et al. (4), and a similar success story in a cardiac allograft model (45) emphasize the importance of T-cell recognition of alloantigens in chronic rejection and advance the hypothesis that blockade of CD28 costimulation is worthy of consideration for its prevention. The experimental evidence is indeed convincing with the caveat that effective early immunosuppression, whether it be targeting of costimulatory molecules or effective drug therapy, might be a fundamental requisite. Indeed, a new immunosuppressive drug, mycophenolate mofetil, an inhibitor of inosine monophosphate dehydrogenase, and recently approved for clinical use, is effective in preventing chronic rejection in the rat renal allograft model (46).

If in fact the initiating signal for chronic rejection is immunologic, can we posit a unifying final pathway for this inexorable disease, and can we find the Achilles heel in the cascade of events leading to chronic rejection? In the rat model, prevention of chronic rejection with CTLA4 Ig was associated with diminished expression of mRNA encoding IL-2, IL-6, IL-12, IFN-γ and tumor necrosis factor α (TNF-α), monocyte chemoattractant protein 1 (MCP-1), and that of transforming growth factor β1 (TGF-β1) (4). In our clinical investigation, intrarenal TGF-β1 mRNA display was a significant correlate of interstitial fibrosis and of chronic rejection of renal allografts (47). In contrast, neither cytotoxic attack molecules (granzyme B, perforin, Fas ligand) nor proinflammatory cytokines (IL-2, IFN-γ) were correlates of chronic rejection (47).

TGF-β1 is a prototypic fibrogenic cytokine implicated in tissue repair/remodeling, and this multifunctional polypeptide has also been identified in the fibrotic scarring of several organs including the kidney (48). Thus, it is tempting to hypothesize TGF-β1 as the nodal point for both antigen-dependent and -independent mechanisms of chronic renal allograft rejection (ref. 47; Fig. 3) as well as consider prevention of TGF-β1 hyperexpression as a prophylactic measure against chronic rejection.

Fig. 1. T-cell/APC contact sites. In this schema of T-cell activation, the antigenic signal is initiated by the physical interaction between the clonally variant TCR α, β heterodimer and the antigenic peptide displayed by MHC on APCs. The antigenic signal is transduced into the cell by the CD3 proteins. The CD4 and the CD8 antigens function as associative recognition structures, and restrict TCR recognition to class II and class I antigens of MHC, respectively. Additional T-cell surface receptors generate the obligatory costimulatory signals by interacting with their counter-receptors expressed on the surface of the APCs. The simultaneous delivery to the T cells of the antigenic signal and the costimulatory signal results in the optimum generation of second messengers (such as calcium) expression of transcription factors (such as nuclear factor of activated T cells) and T-cell growth-promoting genes (such as interleukin (IL) 2). The CD28 antigen as well as the CTLA4 antigen can interact with both the B7-1 and B7-2 antigens. The CD28 antigen generates a stimulatory signal, and the recent studies of CTLA4-deficient mice (56) suggest that CTLA4, unlike CD28, generates a negative signal. CD, cluster designation; LFA-1, leukocyte function associated antigen 1; ICAM-1, intercellular adhesion molecule 1.

elimination, if it occurs in vivo, can create a beneficial hole in the recipient’s graft-destructive T-cell repertory.

Existing data also support suppressor and/or cytokine-based mechanisms of transplantation tolerance (27, 28). The TH1/TH2 paradigm for transplantation, wherein the TH1 cell cytokines IL-2 and interferon γ (IFN-γ) are considered detrimental to allografts, and the TH2 cell cytokine IL-4 is considered tolerogenic, is a provocative concept for transplantation tolerance (29). In support of this model, the CD28/B7 blockade is associated with repression of TH1 cytokines and expression of TH2 cytokines (30). The universality of this principle, however, is tempered by the demonstration that mice, rendered IL-2 deficient by homologous recombination, reject islet allografts (55).

Transplantation tolerance has also been realized in experimental models by additional innovative strategies. In-
FIG. 2. T-cell activation/ergy decision points. Several potential sites for the regulation of T-cell signaling are shown. The antigenic peptide displayed by MHC (site 1), costimulatory signals (site 2), TCR (site 3), and cytokine signaling (site 4) can influence the eventual outcome. Altered peptide ligands, blockade of costimulatory signals, down regulation of TCR, and IL-10 favor anergy induction, whereas fully immunogenic peptides, delivery of costimulatory signals, appropriate number of TCR, and IL-12 (23) prevent anergy induction and facilitate full activation of T cells.

A spectacular success story and quintessential paradigm of translational research—organ transplantation—is a safe (about 90% 1-year recipient survival rate) and efficacious (about 80% 1-year graft survival rate) therapy. However, due to organ shortage, about 10 patients die each day in the United States while awaiting an organ transplant. Thus, we need to fool mother nature, and make every transplanted organ work. Blockade of T-cell costimulation and molecular mechanism-based immunosuppression are indeed attractive avenues.

FIG. 3. Potential mechanisms of chronic allograft nephropathy/rejection. In this schema for chronic rejection, intragraft expression of TGF-β1 represents the nodal point for both antigen-dependent (rejection) and antigen-independent (ischemia, preservation injury, diminished nephron mass (49)) mechanisms. The ability of TGF-β1 to promote its own production (50) (positive feedback loop), its ability to stimulate extracellular matrix accumulation (48), and its additional properties [e.g., induction of platelet derived growth factor production (51), stimulation of production of vasoconstrictor endothelin-1 (52)], are also incorporated into this framework. Also considered are the potential contributions of corticosteroids and cyclosporine, immunosuppressants used in organ transplantation, since these drugs induce TGF-β1 hyperexpression in vitro (53, 54).