The Immune Tolerance Network: The "Holy Grail" Comes to the Clinic

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The concept of reprogramming immune cells in a way that would eliminate pathogenic responses while preserving normal immune function has excited immunologists for decades. Often promised but never fully realized, these highly specific tolerogenic therapies would replace current immunosuppressive regimens and their often debilitating side effects.

It now seems that the more detailed understanding of the molecular events associated with T-cell recognition and activation may provide unprecedented opportunities to test these approaches in humans. As a result of these advances, the Immune Tolerance Network (ITN) was created to identify and promote the use of these tolerogenic therapies in the clinic. Funded by a $144 million contract from the National Institutes of Health (in collaboration with the Juvenile Diabetes Foundation International), the ITN is examining new and innovative therapeutic approaches and bioassays to treat a range of autoimmune diseases, asthma, allergy, and rejection in kidney and islet transplantation.

The Basis of Tolerance

The need for more effective treatments for immune-mediated diseases has never been greater as the incidence of immunologic diseases has increased at an alarming rate. Moreover, kidney, liver, and heart transplantation has become a routine surgical procedure limited only by the shortage of organs and the risk of graft rejection. Over the past 40 years, improved treatment of these diseases has been achieved primarily through the development of increasingly potent nonspecific immunosuppressive drugs to inhibit immune responses (1). Although immunotherapies such as steroids, cyclosporin A, and panreactive antibodies have resulted in considerable clinical success in treating organ transplant rejection, these approaches require life-long treatment and nonspecifically suppress the *entire* immune system, exposing patients to increased risks of infection and cancer. Surprising is that these nonspecific therapies have only a limited beneficial impact on *long-term* graft survival (2). In addition, the drugs are expensive (3) and have limited usefulness in autoimmunity and asthma, where long-term toxicity is not wanted in these usually non–life-threatening diseases. The agents that have permitted the most significant advances in the short-term success of transplants, calcineurin inhibitors, are themselves nephrotoxic. As well, other limiting side effects may occur; for instance, in nondiabetic kidney patients, the combination of calcineurin inhibitors and prednisone is associated with the development of insulin-dependent diabetes in up to 25% of transplant recipients (4,5).

Tolerance therapies, conversely, are selective *short-term* immunotherapies that are targeted toward eliminating only the pathogenic immune response, leaving the beneficial functions untouched. Advances in the development of tolerogenic strategies for transplantation have been fueled by multiple anecdotal cases in which transplant recipients have discontinued all immunosuppression therapies and still not rejected their transplants (6). Although most cases in which immunosuppression has been discontinued result in either fulminate acute or chronic rejection, these successful examples indicate that tolerance is indeed a realistic goal in clinical transplantation and suggest that allograft tolerance using intentionally designed therapies is a viable clinical strategy (7,8).

Such therapies are now a very real possibility as basic immunology research has helped to unravel the fundamental processes that are responsible for immune regulation. In fact, over the past few years, unparalleled progress in our conceptual understanding of the mechanisms at work in the induction of tolerance and the restoration of self-tolerance has led to the development of some unique and selective immunomodulatory strategies that are making their way into the clinic. The demonstration of long-term kidney and islet allograft survival in nonhuman primates that receive anti-CD154 antibody monotherapy (9,10), the new generation of anti–T-cell monoclonal antibodies (mAb) (11,12), the use of bone marrow transplantation to tolerize (13), and selective inhibition of the inflammatory subset of primed autoreactive effector T cells (14,15) are just a few examples. Such accomplishments not only highlight the significant advances made toward effective therapies of immune tolerance but also underscore the breadth of treatment options that are based on the various mechanisms of circumventing autoimmune destruction and that may soon become available to the clinician.

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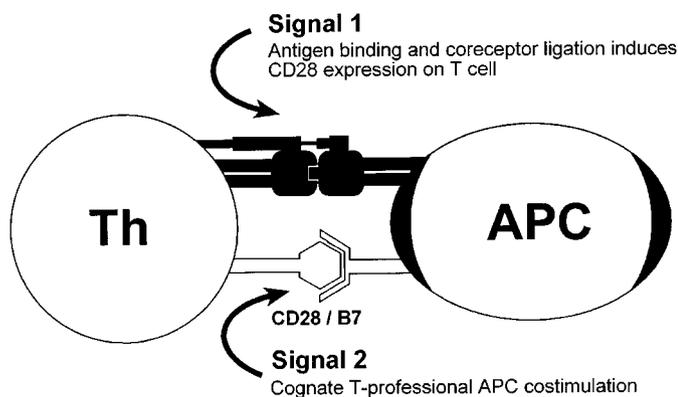
Specific Targets for Immune Intervention

The normal processes that generally are thought to regulate tolerance are based on four mechanisms: clonal deletion, clonal inactivation, cytokine-dependent immune deviation, and suppression (16). Each operates to varying degrees in the generation and maintenance of tolerance, although their relative contribution may vary depending on the nature of the antigen and the location in which "tolerization" occurs.

The primary mediators of immune reactivity in autoimmune, allergy, asthma, and transplant settings are T and B lymphocytes. T lymphocytes have antigen-specific receptors that recognize foreign and self antigens, and B lymphocytes produce antibodies that are reactive against foreign and self tissues. Accessory cells known as antigen-presenting cells (APC) activate naïve T cells by presentation of the antigen together with major histocompatibility complex (MHC) antigens, the primary targets for allorecognition. However, as illustrated in Figure 1, the activation of T and B cells requires signaling not only through the T and B cell receptors (TCR/BCR; Signal 1) but also through co-stimulatory pathways (Signal 2) (17). After activation, a number of cell surface and soluble molecules are known to regulate further the immune response. The number of cell surface molecules that are known to regulate T-cell activation is growing, and more complete descriptions of the molecular actions of proapoptotic molecules such as Fas, Trance, and tumor necrosis factor (TNF) have been compiled (16). Each of these mechanisms provides potential targets for therapeutic interventions, many of which are rapidly nearing clinical application.

Signal 1

An increasing knowledge of the various steps in T-cell activation through the T-cell receptor (TCR) has provided a number of opportunities for intervention. It is now known that the process requires binding of the antigen/MHC complex to the TCR/CD3 complex. This event initiates a cascade of signaling that begins with the activation of several cytoplasmic protein tyrosine kinases. Recruitment of the CD4 (or CD8) co-receptor and its associated tyrosine kinase, Lck, into the vicinity of the TCR complex is believed to induce phosphorylation of CD3 proteins, which ultimately leads to downstream signal progression. *In vitro* studies have suggested that interruption of this signaling pathway at a number of points may lead to tolerance. Thus, mAb that are directed at the TCR and co-receptor molecules, altered TCR ligands, and MHC-derived peptides present novel approaches to tolerance induction. A number of these are ready to be applied clinically. Promising drugs include nonmitogenic anti-CD3 mAb, anti-CD4 mAb, systemic and oral peptide therapies (Copaxone and MHC peptides), Campath-1H (anti-CD52), and DNA vaccination for allergy therapy, *e.g.*, ragweed. One of the most successful strategies for tolerance induction in nonhuman primate renal transplantation has been depletion of immune cells at the time of the transplantation. Gradual repopulation of the immune cells occurs after the organ transplantation, often in the absence of inflammation and associated rejection. Pilot studies have been initiated in transplant recipients to test these new drugs in humans. As an example, nonmitogenic anti-CD3 mAb have been used in several phase I kidney transplant trials with similar efficacy as the original mitogenic OKT3 mAb without the severe associated side effects (11,18). Similar early clinical studies have been performed with Campath 1 (19). Although it is early in the study, the results are promising and may represent an alternative approach to the development of tolerance.

Signal 2

The additional co-stimulatory signal required for complete T-cell activation, cell cycle progression, survival, and maximum effector function presents a number of additional therapeutic targets. Candidate co-stimulatory molecules are under investigation for tolerance induction. Some of these are soluble factors, such as interleukin-2 (IL-2) and IL-12, and many are T-cell surface receptors, such as CD28, lymphocyte function-associated antigen-1, 4-1BB, CD2, CD30, CD44, and CD40 ligand (CD40L). Each has the ability to augment the T-cell proliferative response to antigenic stimuli. It is likely that each of these acts through different mechanisms, some delivering co-stimulatory biochemical signals to the T cell, some enhancing adhesion to antigen-presenting cells, and still others mediating homing to target tissues. Specifically, blockade of T-cell co-stimulatory targets CD28 and CD40L during TCR engagement has been shown to induce a state of antigen-specific tolerance (20). Prevention of CD28/B7 and CD40L/CD40 interactions with anti-CD40L and CTLA4Ig during kidney transplantation in monkeys results in greatly prolonged allograft survival without the need for other immunosuppressive drugs (21). Additional approaches may include alloantigen pretreatment, *e.g.*, donor-specific transfusion, under the cover of short-term immunotherapy with anti-CD154, CTLA4Ig (anti-B7 mAb), or nondepleting anti-CD4 mAb. Furthermore, the CD2 pathway has repeatedly been found to exert a major role in human immune responses, out of proportion to its effects in rodents (which lack lymphocyte function-associated antigen-3 and only engage CD2 with CD48). Finally, combined targeting

Signal 1
Antigen binding and coreceptor ligation induces CD28 expression on T cell

Th   **APC**

CD28 / B7
Signal 2
Cognate T-professional APC costimulation

Figure 1. T-cell activation requires signaling through the T-cell receptor (TCR) (Signal 1) and co-stimulatory pathways (Signal 2). For example, antigen/MHC binding to the TCR and CD28 interaction with B7.1 or B7.2 expressed on the surface of the antigen presenting cells (APC). Both of these signal pathways allow opportunity for intervention in immune-mediated diseases.

of Signal 1 and Signal 2, *e.g.*, nonmitogenic anti-CD3 plus B7 or CD40L blockade, should result in a synergistic effect on tolerance induction. These therapies are some of the most promising and exciting candidates for clinical tolerance induction in transplantation and autoimmune diseases.

Targeting Clonal Inactivation/Deletion

It is clear that the least pathogenic T cell is a dead T cell. Thus, multiple therapeutics have been developed to promote clonal deletion either centrally within the thymus or in the periphery. One approach that can be applied clinically is the use of autologous or allogeneic bone marrow transplantation to "re-educate" the immune response and promote deletion of autoreactive cells in the thymus. In addition, mixed chimerism with nonmyeloablative conditioning holds promise for the induction of transplant tolerance. This approach has been shown to achieve reliable and robust tolerance in rodents, and recent studies in large animals and humans show that mixed chimerism can also be induced with tolerable, nonmyeloablative conditioning (22). Regimens that combine extrathymic T-cell deletion (using mAb) followed by central T-cell tolerance induced by donor bone marrow under the cover of combined co-stimulatory blockade and low-dose total body irradiation are in the planning stage. Another, distinct approach uses proapoptotic therapies that take advantage of the Fas, TNF, and Trance pathways to promote activation-induced programmed cell death to eliminate antigen-stimulated T cells. These therapies can be combined with other T-cell inhibitors, such as anti-CTLA-4 agonists, to shut down the ongoing immune responses and promote tolerance. In more direct approaches, cytokines, such as transforming growth factor-β, IL-10, and others, have been shown broadly to suppress pathogenic T cells, whereas IL-4 and interferon-γ can alter the balance of Th1 and Th2 responses (immune deviation) that can be used to regulate asthma, allergy, transplantation, and autoimmunity (23).

Immune Tolerance Network

Because of these advances, the National Institute on Allergy and Infectious Diseases (NIAID) convened an Expert Panel on Immune Tolerance in the fall of 1997 to determine how best to capitalize on the extraordinary opportunities emerging. The result of this process was a multiyear contract for the establishment of the ITN, aimed at developing a new, comprehensive approach to the *clinical application* of immune tolerance. The contract was awarded in October 1999 by the NIAID, with additional funds provided by the National Institute for Diabetes and Digestive and Kidney Disease and the Juvenile Diabetes Foundation International.

The effort is a collaborative research program that spans a broad platform of diseases, providing a long overdue recognition of the common fundamental bases of tolerance among a diverse set of human conditions. The ITN mandate spans research in kidney and islet transplantation, autoimmune disease, and allergy and asthma. In addition to seeking viable clinical applications in these diseases, the ITN encompasses an effort to define further the clinical parameters of tolerance

induction through a complementary mechanistic study and bioassay research program.

Clinical trials and mechanistic studies adopted by the ITN are generated through an investigator-driven process that is open to all investigators who are interested in immune tolerance. The two-step application process, designed to allow fast and efficient peer review of submitted proposals, begins with the submission of a short "concept proposal" through the ITN web site (www.immunetolerance.org). Complete review of all proposals takes approximately 3 mo, and there are two funding cycles each year. Initially, the ITN is primarily seeking proposals for Phase I, II, or III trials of novel therapies that have a strong biologic basis for inducing tolerance and for which preclinical investigation is complete, with encouraging results. Of particular interest are trials that combine existing agents that have not yet been tested together. Proposals for tolerance assays or mechanistic studies should complement the clinical portion of the research and may either be of broad clinical application or disease or be protocol specific.

Research in each disease area is managed by a team of specialists, or subgroups, in each disease area (Figure 2). The ITN has already completed three review cycles and has begun developing clinical protocols in each of its targeted disease areas.

Kidney Transplantation

Although the Kidney Transplantation subgroup is committed to developing protocols that will promote tolerance induc-
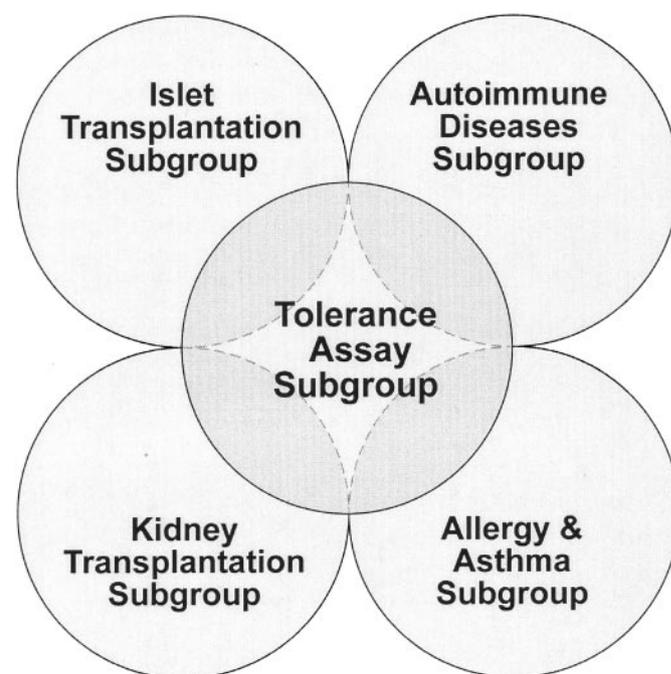

Figure 2. Each of the clinical subgroups is charged with developing and managing clinical trials within one of the Immune Tolerance Network's targeted disease areas. Each of these subgroups works closely with the Tolerance Assay Subgroup to develop a coordinated set of mechanistic studies and bioassays for monitoring the development, maintenance, and loss of tolerance in each clinical trial.

tion in kidney transplant recipients, the high success rate of current immunosuppressive protocols places some limits on the freedom to develop proactive, potentially tolerogenic strategies. The serious side effects associated with existing protocols, however, justify the need for these more specific therapies. The Kidney Transplantation Subgroup of the ITN has prioritized the following concepts for study: (*1*) bone marrow transplantation trials for patients with multiple myeloma, (*2*) co-stimulatory blockade regimens, and (*3*) calcineurin inhibitor-sparing or free regimens. The first proposals have focused on myeloablative strategies coupled with donor cell (including stem cell/bone marrow/peripheral blood mononuclear cell) transplant along with the allogeneic kidney. Another avenue being pursued by the ITN is the development of a network of tolerant patients (both liver and kidney) for detailed mechanistic analyses to increase our understanding of the tolerant state. Finally, efforts are being made to develop a calcineurin-free therapy for kidney transplant recipients in the hope of producing robust tolerance, and a protocol using rapamycin instead of cyclosporin A or FK506 is under consideration.

Islet Transplantation

The Islet Transplantation Subgroup is focused on developing safe and successful strategies for restoration of self-tolerance and induction of donor-specific allotolerance in islet allotransplantation for type 1 diabetes. The first trial initiated by the ITN will be led by Dr. James Shapiro of the University of Alberta to conduct a multicenter trial of solitary islet transplantation in type 1 diabetic patients using a novel protocol. The "Edmonton Group" has achieved exceptional success in achieving sustained insulin independence in multiple type 1 diabetic patients. In the proposed trial, c-peptide–negative type 1 diabetic patients with failure of insulin therapy will undergo solitary islet transplantation by a minimally invasive percutaneous transhepatic intraportal approach and will be immunosuppressed using a combination of daclizumab (anti–IL-2 receptor) induction with low-dose tacrolimus and sirolimus. Fresh islets derived from a maximum of two cadaveric donors, providing at least 10,000 islet equivalents, will be transplanted after minimal cold storage and without culture. Insulin therapy will be withdrawn provided blood glucose is maintained at less than 10 mmol/L. The ITN will support 40 transplants at 10 centers in this Phase II study. In addition, the ITN will support metabolic studies at all participating centers to ensure that a detailed analysis of islet cell quality and function can be determined to provide critical data for the second phase of the studies.

Building on the unprecedented success reported using the Edmonton protocol, the group will seek to establish new standards for human islet preparation and quality control. The ITN will identify, validate, and implement immune monitoring assays tailored to the specific challenges posed by islet transplantation in autoimmune diabetes mellitus. Successful completion of the proposed multicenter trial, the first of its kind in the history of islet transplantation, will provide a platform for future tolerance induction protocols in islet transplantation. Other protocols of interest to this group include donor/antigen

cell pretreatment under cover of short-term co-stimulatory or co-receptor blockade, mixed chimerism without myeloablation, and anti-CD3 immunotoxin.

Autoimmune Diseases

The ITN will conduct clinical trials and mechanistic studies aimed at alleviating the immune responses that underlie type 1 diabetes mellitus, systemic lupus erythematosus (SLE), rheumatoid arthritis, multiple sclerosis, and other autoimmune diseases. The Autoimmune Diseases Subgroup is evaluating the most appropriate concepts and disease settings for trials within the group's mandate. These fall into antigen-specific and nonspecific categories. Initial trials using anti–T-cell mAb are being entertained to determine whether nondepleting dosages of newer antibodies can alter surrogate markers of disease progression and whether anti–T-cell antibodies synergize with therapies that block co-stimulation or neutralize proinflammatory cytokines. There is considerable evidence from animal models of both multiple sclerosis and SLE to indicate that the B7/CD28 pathway plays an important role in autoreactivity, and, as such, early stage clinical studies of blockade of the B7/CD28 pathway will therefore be prioritized by the ITN.

An early trial approved by the ITN involves CTLA4Ig therapy in SLE. This study seeks to determine whether treatment with CTLA4Ig alone or in combination with cyclophosphamide (CTX) can induce remission more effectively than CTX alone in severe lupus nephritis. The investigators have presented preclinical evidence that administration of CTLA4Ig together with CTX can lead to improvement in renal function and renal histopathology in NZB/W mice with active lupus (D.I. Daikh and D. Wofsy, personal communication, May 2000). Safety and efficacy of CTLA4Ig in nonimmunocompromised humans has been demonstrated in a phase I trial of psoriasis, and further safety information will be obtained from a trial of CTLA4Ig in rheumatoid arthritis by the drug's manufacturer. Approximately 100 patients will be enrolled in the trial at clinical centers around the United States.

The ITN is also developing a novel anti-CD3 therapy for type 1 diabetes mellitus. A Phase I toxicity trial in progress has shown no evidence of systemic toxicity or commonly observed side effects. Approximately 30 individuals will be treated and followed for 24 mo to evaluate disease progression.

In addition to these trials, the ITN is actively seeking novel applications of soluble mediators such as IL-4, IL-10, transforming growth factor-β and anti-IL-10/12, anti-TNF, or anti-interferon-γ antibodies in clinical scenarios not currently duplicated by the pharmaceutical industry.

Allergy and Asthma

The goal of the immunologic interventions studied by the Allergy & Asthma Subgroup is to ameliorate allergen-specific Th2-driven processes and replace these processes with allergen-specific, protective immune responses. Among the diseases that fall under the research mandate of the Allergy & Asthma Subgroup are allergic rhinitis (seasonal and perennial) and allergic asthma. The future focus for treating allergies/asthma moves beyond symptomatic therapies, developing

treatments that alter the underlying immune system, and developing protective immunity. Protective immunity consists of allergen-specific immune deviation and allergen-specific tolerance induction.

The ITN is working with investigators to develop a protocol for the treatment of allergic rhinitis using unique sequences of DNA that have been found to have immunostimulatory activity (ISS). Allergens that are chemically linked to ISS can be injected into the skin at 100-fold higher amounts than natural allergen, without causing an allergic reaction. In animal models of allergic disease, allergen linked to ISS has been found to be more effective than the allergen extracts currently used clinically. The planned study will be the first clinical trial in which ragweed allergen linked to ISS is used for immunotherapy in ragweed-allergic patients with hay fever.

Tolerance Assays

In each of the disease areas, the ITN's Tolerance Assay Subgroup (TAG) will support the clinical trials by (*1*) examining the mechanisms that create and maintain the tolerant state and (*2*) developing a set of assays that will function as a road map to guide clinicians in their attempt to create and monitor immune tolerance in individual patients. Initially, the TAG is developing studies aimed at establishing baseline measurements of a number of biomarkers for clinical trials in transplantation. The TAG is also developing several core facilities and working to standardize assays for use at individual clinical centers. ITN core facilities are being set up for PCR-based gene expression and polymorphisms, pharmacogenomics and microarrays, MHC peptide complexes, and cell-based tolerance assays. Cytokine enzyme-linked immunosorbent assays, antibody detection, pharmacokinetics, and flow cytometric assays, *e.g.*, insulin, GAD45, and IA-2 for diabetes, are being standardized for more universal application. For each patient enrolled in ITN trials, a battery of measurements will be performed and tailored to the clinical protocols under investigation. In addition, the ITN is establishing procedures and facilities for banking of clinical materials obtained in all sponsored trials for use in ongoing tolerance assay and biomarker studies, as well as for retrospective studies of emerging markers.

Looking Ahead

The ITN is striving to develop a new paradigm in clinical research of immune-mediated diseases. Through an open and inclusive framework of investigator-driven research, a broad disease-based focus, a strong government and regulatory interface, and mutually beneficial collaborations with industry, the ITN hopes to advance our ability to create and maintain clinical tolerance and further define the underlying mechanisms at work in the induction and loss of tolerance. Although the project is still in its infancy, the ITN is looking forward to when chronic therapies will yield true cures for immune-mediated diseases.

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