

Molecular mechanisms in allergy and clinical immunology

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The Immune Tolerance Network: A new paradigm for developing tolerance-inducing therapies

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Immune tolerance therapies are designed to reprogram immune cells in a highly specific fashion to eliminate pathogenic responses while preserving protective immunity. A concept that has tantalized immunologists for decades, the development of tolerance-inducing therapies, would revolutionize the management of a wide range of chronic and often debilitating diseases by obviating the need for lifelong immunosuppressive regimens. The advances of the past decade have provided a more detailed understanding of the molecular events associated with T-cell recognition and activation. Building on these advances, immunologists have demonstrated the feasibility of various tolerance-inducing approaches in small- and large-animal models of autoimmunity, allergy, and transplant graft rejection. Unprecedented opportunities to test these approaches in a variety of human diseases have now emerged. To capitalize on these advances, the National Institutes of Health recently established the Immune Tolerance Network (ITN), an international consortium of more than 70 basic and clinical immunologists dedicated to the evaluation of novel tolerance-inducing therapies and associated studies of immunologic mechanisms. By using a unique interactive approach to accelerate the development of clinical tolerance therapies, the ITN is partnering with the biotechnology and pharmaceutical industries to examine innovative tolerogenic approaches in a range of allergic and autoimmune diseases and to prevent graft rejection after transplantation. Two years since its inception, the ITN now has approximately 2 dozen clinical trials or tolerance assays studies ongoing or in later stages of protocol development. This report summarizes the rationale for emphasizing clinical research on immune tolerance and highlights the progress of the ITN. (*J Allergy Clin Immunol* 2002;110:17-23.)

Key words: Immune tolerance, costimulation, anergy, autoimmunity, transplantation, asthma, allergy

Abbreviations used

APC: Antigen-presenting cell
ITN: Immune Tolerance Network
TCR: T-cell receptor

THE IMMUNOLOGIC BASIS OF IMMUNE TOLERANCE

Immune-mediated diseases and immune-mediated transplant rejection collectively affect tens of millions of Americans and result in high medical and social costs. Over the past 40 years, advances in the treatment and prevention of transplant rejection have been achieved largely through the development of increasingly potent but globally immunosuppressive drugs. Systemic glucocorticosteroids, calcineurin inhibitors, antimetabolites, purine synthesis inhibitors, and panreactive mAbs have met with a degree of clinical success in treating acute transplant rejection.¹ However, such therapies generally require life-long use, are costly,² and are associated with serious side effects, including nephrotoxicity, diabetes, and immunosuppression, exposing patients to heightened risks of infection and malignancy. Surprisingly, these agents have had only a modest effect on chronic graft rejection and long-term graft survival and have had limited utility in the management of autoimmune diseases and asthma and allergy.

Hence a major goal of modern clinical immunology is to develop new strategies and treatments that will induce a state of immune tolerance by selectively blocking or eliminating pathogenic immune responses while maintaining protective immunity. Enthusiasm for the development of tolerizing therapies has been fueled by many anecdotal cases in which transplant recipients have discontinued immunosuppressive medications and maintained functioning grafts without evidence of rejection.³⁻⁵ The prospects for tolerizing therapies are now quite promising because research into basic immunology has helped to unravel the fundamental processes responsible for self-tolerance and immune regulation. A variety of agents and approaches to induce or restore immune tolerance are now entering clinical trials and, if successful, will find applications in a

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range of clinical scenarios, spanning allergy and autoimmunity in addition to transplantation.

During immune development, the thymus molds the developing T-cell repertoire centrally by means of deletion of self-reactive clones. Small numbers of autoreactive T cells escape to the periphery, either through incomplete negative selection or because not all peripheral antigens are displayed within the thymus. These self-reactive T cells are inactivated at extrathymic sites, primarily the lymph nodes and spleen, when mature T cells encounter self-antigens. The processes that regulate peripheral tolerance (ie, clonal inactivation, clonal deletion, and cytokine-dependent suppression and immune deviation) operate, to varying degrees, in the generation and maintenance of tolerance, although their relative contributions might vary depending on the nature of the antigen and the location in which tolerization occurs (Fig 1).⁶ The existence of multiple pathways presents a wide range of potential targets for intervention. Indeed, dozens of ligands, receptors, and signaling intermediates provide the structural underpinnings for a host of candidate drugs. This complexity also introduces many practical challenges because of the potential for functional redundancies in the targeted pathways and heterogeneity in their expression among different diseases and affected individuals.

TARGETS FOR INTERVENTION

Signal 1 and signal 2

Naive T cells require 2 distinct signals to become fully activated (Fig 2). Signal 1 is propagated on presentation of antigen to the T cell, initiating a signaling cascade involving a number of molecules, including the CD4 or CD8 coreceptors and their associated kinases. Professional antigen-presenting cells (APCs) deliver additional costimulatory signals, termed signal 2, that elicit robust and durable T-cell responses. Costimulation is required for complete T-cell activation, whereas propagation of signal 1 in the absence of signal 2 leads to aborted T-cell responses, anergy, or death.⁷ Thus costimulatory pathways present rich opportunities for intervention, with the significant benefit that a priori knowledge of the target antigen might not be necessary.

The most extensively studied of the costimulatory pathways involves the APC proteins CD80 (B7-1) and CD86 (B7-2) and their T-cell receptor (TCR), CD28.^{8,9} Other APC proteins, including CD40, 4-1BB ligand, and a molecule called LIGHT, provide costimulation through their TCRs, CD40 ligand, 4-1BB, and HVEM (or herpes virus entry molecule), respectively.¹⁰⁻¹² These receptors act either directly in a costimulatory fashion or by upregulating other receptors and ligands needed for generation of signal 2, including CD28 itself. A major effect of costimulation is production of IL-2 and other cytokines required for T-cell proliferation and for arming differentiated T cells to take on effector functions. Once fully differentiated and armed for effector functions, neither CD4⁺ nor CD8⁺ T cells require costimulatory signals to respond. Signal 1 is sufficient to stimulate secondary responses.

Interrupting signal 1 at a number of points might lead to tolerance, which might be antigen specific depending on the approach (eg, by mAbs directed at the TCR and coreceptor molecules, by MHC-derived peptides through presentation of altered TCR ligands, or through alloantigen pretreatment when donor-specific transfusion is combined with solid organ transplantation).¹³ Several of these have been or are ready to be applied clinically. Promising candidates include nonmitogenic anti-CD3 mAb and CD4 mAbs, anti-CD52 mAb (Campath-1), systemic and oral peptide therapies (Copaxone and MHC peptides), and peptide-based immunotherapies for asthma and allergy.

Costimulatory blockade with anti-CD40 ligand greatly prolongs renal allograft survival in nonhuman primates without the need for other immunosuppressives. For example, monkeys infused monthly with anti-CD40 ligand remained rejection free and maintained functioning allografts for up to 2 years.¹⁴ Similarly encouraging results were achieved in pancreatectomized rhesus monkeys that received islet transplants under cover of anti-CD40 ligand alone.^{15,16} However, in neither of these experimental systems did the animals become fully tolerant. Future studies targeting both signal 1 and signal 2 (eg, with nonmitogenic anti-CD3 plus anti-CD80/86 or anti-CD40 ligand) might result in synergistic effects on tolerance induction. Such therapies represent some of the most promising approaches in transplantation and autoimmune diseases.

Clonal deletion

Multiple approaches are being pursued to promote clonal deletion, either centrally within the thymus or in the periphery. T cell-depleting antibodies and immunotoxin conjugates have been given at the time of transplantation, establishing a window for regeneration of the T-cell repertoire in the presence of alloantigen. This approach leads to long-term graft survival and perhaps true tolerance in rodent and nonhuman primate models.¹⁷⁻²⁰

Another approach that is being applied in large-animal models clinically involves combining renal transplantation with myeloablation and allogeneic bone marrow transplantation.^{21,22} The resulting bone marrow chimerism leads to immune reconstitution characterized by central deletion of graft-reactive cells, with robust tolerance in rodent and some large-animal models. Other approaches take advantage of the Fas, TNF, and Trance pathways to promote activation-induced cell death. T cells become susceptible to activation-induced cell death on repetitive stimulation as a result of upregulation of IL-2 and cell-surface death receptors, primarily Fas.²³ Another form of T-cell apoptosis, termed death by neglect, occurs when activated T cells are deprived of growth factors, such as IL-2 and other cytokines. One well-studied pathway involves the CD28 homologue CTLA4, which also binds CD80/86 but with a higher affinity than that of CD28. CTLA4 acts as a competitive inhibitor of CD28, blocking CD28-mediated clonal expansion and triggering cell-cycle arrest through downregulation of IL-2. Gene knockout experiments highlight the importance of these mechanisms in immune homeostasis because

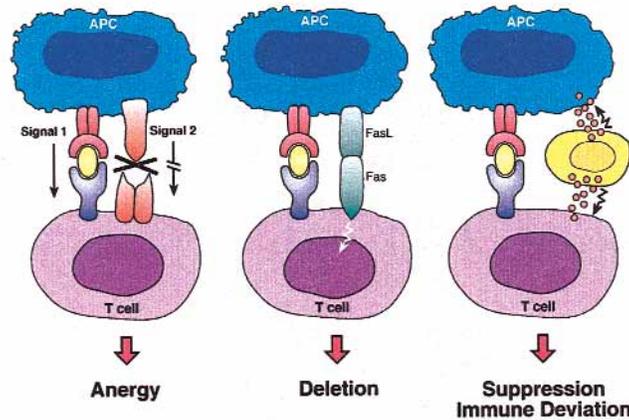


FIG 1. Molecular basis of immune tolerance: schematic diagram of molecular pathways of tolerance induction. *Left panel, Anergy.* T cells that receive signal 1 in the absence of signal 2 (costimulatory blockade) become nonresponsive or anergic and remain anergic on subsequent antigen-specific stimulation, even in the presence of signal 2. A number of other approaches that interrupt signaling through the TCR might also lead to anergy (see text). *Center panel, Deletion.* Activated T cells upregulate the expression of Fas; subsequent binding of Fas ligand induces T cells to undergo apoptotic cell death. Fas ligand might be expressed on APCs, activated T cells, and stromal cells of immunologically privileged sites. *Right panel, Suppression and immune deviation.* Stimulated T cells might be tolerized through direct cell-cell contact (suppression) or soluble factors derived from regulatory T cells (immune deviation).

fatal lymphoproliferative syndromes and autoimmunity develop in Fas- and CTLA4-deficient mice.²⁴

Immune deviation and suppression

These forms of peripheral tolerance are characterized by downregulation of stimulated T cells by soluble factors (immune deviation) or by direct cell-to-cell contact (suppression). Cytokines, such as transforming growth factor β , IL-10, and others, might broadly suppress pathogenic T cells, whereas IL-4 and IFN- γ can alter the balance or character of T_H1 and T_H2 responses. Certain indirect means to alter the cytokine environment during antigen presentation appear promising in animal models (eg, by delivering allergen in the presence of immunostimulatory DNA, driving allergen-specific responses in a T_H1 direction).²⁵

THE IMMUNE TOLERANCE NETWORK

This steady stream of advances led the National Institute of Allergy and Infectious Diseases to emphasize research on immune tolerance through nearly a dozen research solicitations beginning in the late 1990s. The Immune Tolerance Network (ITN), the largest of these efforts, is an international consortium dedicated to the clinical evaluation of promising agents for the induction and maintenance of immune tolerance. Established in 1999, the ITN receives additional support from the National Institute for Diabetes and Digestive and Kidney Disease and the Juvenile Diabetes Research Foundation.

The Network's mandate is to conduct clinical trials of new therapies aimed at producing stable, long-term immune tolerance for asthma and allergy, islet and kidney transplantation, and autoimmune diseases. In addition to studying the safety and efficacy of new approach-

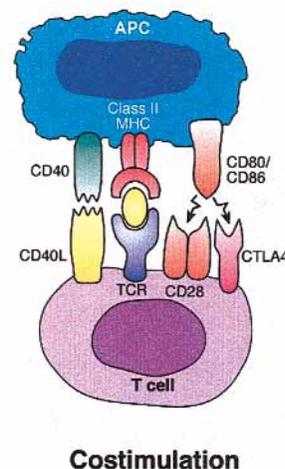


FIG 2. Molecular interactions leading to T-cell activation. Schematic diagram of the antigen-specific (signal 1) and costimulatory (signal 2) interactions between an APC and the T cell. Signal 1 depends on interactions of the MHC-peptide complex with the TCR. Signal 2 is illustrated here by interactions of CD80 or CD86 with CD28. In contrast, CTLA4 acts as a competitive inhibitor of CD28, blocking CD28-mediated events.

es, the ITN mission includes exploration of the underlying mechanisms of tolerance induction and the development and validation of biomarkers to monitor the induction, maintenance, and loss of clinical tolerance in human subjects.

The ITN membership includes more than 70 internationally recognized basic and clinical investigators representing approximately 45 academic institutions and companies in the United States, Canada, Western Europe, and Australia. The ITN organizational structure includes subcommittees dedicated to each of the disease areas within

TABLE I. ITN core facilities

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|---|
| • Gene Microarray Facilities: University of California-San Francisco/Affymetrix, Cornell, Harvard |
| • Real-Time PCR: Celera |
| • MHC-Multimers (Class I and II): University of California-San Francisco, University of Washington, Harvard, University of Colorado |
| • Elispot: Case Western University |
| • TCR profiling: Nantes, France |
| • Infectious diseases-virology: Berlin, Germany |
| • Tissue sample repository: McKesson Bioservices; sample acquisition, preparation, storage, and distribution |
| • Flow cytometry: Distribution of software and standardized reagents for on-site use |
| • Autoantibody Core: In development |
| • Tissue analysis: In development |

the ITN mission, as well as cross-disciplinary committees responsible for the development of tolerance assays, scientific peer review, policy and ethics, publications and communications, and government and industry liaison. ITN membership, committee chairs, and contact information are posted on the ITN Web site, www.immune-tolerance.org.

The ITN seeks proposals for phase I and II trials of novel therapies that have a strong biologic basis for inducing tolerance and for which preclinical investigation is complete, with encouraging results. In addition to trials of single agents, the ITN is interested in studies that combine licensed or investigational agents that have not yet been tested together or novel approaches, such as combined bone marrow and solid organ transplantation. The ITN also accepts applications for biologic assays and biomarkers that might be of either broad clinical application in tolerance research or disease and protocol specific.

The projects the ITN chooses to pursue are the product of a year-round investigator-initiated application process open to all investigators with an interest in immune tolerance. This takes place in 2 stages designed to streamline peer review and to provide a more interactive process than is typical of National Institutes of Health study sections. ITN peer review seeks not simply to critique, accept, or reject a given proposal but to work with investigators in a highly interactive fashion to develop a comprehensive research plan with the greatest chance of success. Initially, a 2-page concept proposal is submitted online, focusing on the rationale and conceptual background for the proposed work. Proposals accepted for further consideration can be developed solely by the applicant, in collaboration with the relevant ITN subcommittee (eg, asthma-allergy, islet or kidney transplantation, autoimmune diseases, or the ITN tolerance assay group), or with input from the ITN Statistical and Clinical Coordinating Center. At this stage, proposals are subjected to detailed review, including presentation in person by the principal investigator and industry cosponsors. This reiterative format provides constructive oppor-

tunities to present new data and respond directly to concerns raised in the review. The ITN has placed a high priority on its ability to respond to late-breaking discoveries; complete review of proposals takes approximately 3 months, allowing multiple funding cycles each year. In the 2 years since its inception, the ITN has reviewed more than 150 concept proposals, and nearly 50 of these have been further developed into full proposals, including approximately 2 dozen clinical trials and tolerance studies that have been selected for implementation. The majority of proposals to the ITN and of those selected for implementation represent efforts of investigators not initially affiliated with the ITN.

The inclusive and interactive nature of the ITN is maintained throughout the protocol-development process. For tolerance researchers, collaboration with the ITN means more than research funding alone. During the protocol-development stage, the ITN works with the investigators to develop a set of integrated tolerance assays and mechanistic studies that complement the clinical aspects of the research. The ITN also provides a wealth of assistance in protocol development, clinical and regulatory expertise, and administrative support. Through its Tolerance Assay Subgroup, the ITN supports various state-of-the-art core facilities with the capacity to dissect the biologic underpinnings of tolerance (Table I). Some of these, such as the Gene Microarray Core and the Real-Time PCR Facility offer investigators access to tools that might be too expensive or labor intensive for individual laboratories. Others, such as the Single Nucleotide Polymorphism Core, the ELISPOT Core, the MHC-Peptide Multimer Core, and the Infectious Diseases Core, offer centralized and standardized methodologies that allow comparisons across multiple treatment settings. In addition, the ITN maintains a clinical sample repository for materials derived from all ITN-sponsored trials. Data generated by the clinical and core laboratories will be linked through computational platforms with the capacity for sophisticated analyses, cross-trial comparisons, data mining, mechanistic model building, and hypothesis generation. This combination of an open and interactive structure, a cross-disciplinary research mandate, a focus on underlying mechanisms, access to state-of-the-art research facilities, and a broad base of world-class expertise make the ITN unique within academia and industry.

CURRENT FOCUS

Asthma and allergy

Advances in pharmacotherapy have greatly improved the health and quality of life for the majority of patients with asthma and allergic diseases. Nonetheless, there remains a significant group of patients, especially those with seasonal allergic rhinitis, who respond poorly to these treatments and for whom allergen immunotherapy is currently recommended. In this sense the focus of ITN in asthma and allergy is unique because successful allergen immunotherapy has many of the hallmarks of tolerance induction (eg, long-term immune modulation and clinical

benefits after cessation of immunotherapy, as demonstrated in bee venom hypersensitivity²⁶ and seasonal allergic rhinitis²⁷). In contrast to many of the other diseases studied by the ITN, asthma and allergy offer unique opportunities on the basis of (1) availability of patients and ease of achieving enrollment targets; (2) perceived safety of immunotherapy in carefully controlled clinical and research settings; (3) relative ease of access to relevant cells and tissues (eg, nasal lavage and biopsy); (4) knowledge of the relevant antigens, many of which are available in recombinant forms, and their B- and T-cell epitopes; and (5) a wealth of preliminary data on potential biomarkers of disease, response to therapy, and immune phenotypes of naturally tolerant cohorts (eg, serum IgE, IgG isotypes, and cytokine expression profiles).²⁸

Hence a major objective of the ITN is to integrate the tools of modern molecular immunology into the practice of allergen immunotherapy. In so doing, it should be possible to shorten the time required to achieve beneficial and long-lasting immune responses; selectively amplify responses, making immunotherapy more robust and durable; and bring greater safety to the clinical practice of immunotherapy. With these and other advances, immunotherapy might be more widely applied in additional settings, such as food allergy, in which safe and effective treatments are currently lacking. Furthermore, recent studies have demonstrated the effectiveness of immunotherapy in preventing or delaying the development of asthma in a cohort of children given immunotherapy for allergic rhinitis, suggesting that additional medical indications might accompany advances in these areas.

Ongoing ITN-sponsored trials or those in protocol development include the following:

- Ragweed allergen desensitization for seasonal allergic rhinitis with a chemical conjugate of ragweed allergen Amb a 1 and oligonucleotide DNA immunostimulatory sequences (investigators: D. Broide and P. Creticos). These unique sequences of DNA promote allergen-specific, T_H1-like responses; are highly effective in preventing and modifying established disease in murine models of asthma; and can be injected intradermally at approximately 100-fold higher levels than natural allergen, without causing an allergic reaction.
- Ragweed allergen desensitization with a chemical conjugate of ragweed allergen Amb a 1 and oligonucleotide DNA immunostimulatory sequences (investigator: D. Broide). The objectives of this study are similar to those listed above but focus on a cohort of West Coast subjects not naturally exposed to ragweed allergen.

Kidney transplantation

Despite the success of modern immunosuppressives, the need for tolerogenic therapies is made amply clear by the toxicities of current regimens and their inability to substantially alter rates of chronic rejection and long-term graft survival. The ITN places a high priority on the following concepts for study: (1) bone marrow microchimerism to induce tolerance in solid organ trans-

plantation; (2) costimulatory blockade regimens; (3) calcineurin inhibitor-sparing or inhibitor-free regimens; and (4) development of a registry-repository of spontaneously tolerized patients for detailed mechanistic analyses to increase understanding of the tolerant state.

Ongoing ITN-sponsored trials or those in protocol development include the following:

- Multisite trial of combined bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease (investigator: M. Sykes).
- Combined bone marrow and kidney transplantation for end-stage renal disease (investigators: D. Sachs and A. Cosimi).
- Anti-CD52 mAb and sirolimus withdrawal in kidney transplantation (investigator: S. Knechtle).
- Antithymocyte globulin, lymphoid irradiation, and CD34⁺ bone marrow transplantation in renal transplantation (investigator: S. Strober).
- Anti-CD3 mAb and sirolimus in kidney transplantation (investigator: H. Kreis).

Islet transplantation

The success of the Edmonton Protocol has reinvigorated international efforts in islet transplantation. As reported nearly 2 years ago, this glucocorticosteroid-free immunosuppressive regimen led to high rates of insulin independence and major improvements in quality of life for a small cohort of insulin-dependent, brittle diabetics.²⁹ The ITN is seeking to replicate these results in a phase II multisite international trial to (1) assess site-to-site reproducibility, (2) institute new standards for human islet preparation and quality control, (3) foster development of an international network of sites capable of conducting carefully controlled islet transplants, and (4) provide a platform for the evaluation of future tolerance-induction strategies in islet transplantation. The ITN also places a high priority on islet transplantation trials by using potentially tolerogenic approaches plus low-dose immunosuppression, with the intention of weaning patients from immunosuppressives in a carefully controlled manner.

Ongoing ITN-sponsored trials or those in protocol development include the following:

- ITN Edmonton Protocol: cadaveric islet transplantation through transhepatic percutaneous portal vein infusion. Forty brittle diabetics at 10 sites in the United States, Canada, and Europe will receive 2 transplants (required to provide an adequate islet mass) plus induction therapy with anti-IL-2 receptor and low-dose tacrolimus and sirolimus (principal investigator: J. Shapiro).
- Anti-CD3 with sirolimus withdrawal in islet transplantation: cadaveric islet transplantation through transhepatic percutaneous portal vein infusion (investigator: B. Hering). This potentially tolerizing regimen builds on the success of the Edmonton approach and incorporates additional measures to enhance islet engraftment, survival, and function. Among these are (1) ex vivo culture of harvested islets before transplantation, allowing for future modifications, such as

exposure to islet growth factors and genetic manipulations to enhance islet acceptance, and (2) pretransplant induction with a non-T cell-activating humanized monoclonal anti-CD3. In pilot studies a single islet transplant under these conditions provides an adequate islet mass to achieve insulin independence. If successful, at approximately 1 year after transplantation, subjects with biomarkers of donor-specific tolerance will be randomized to maintenance immunosuppression versus sirolimus withdrawal.

- Anti-CD52 with sirolimus withdrawal in islet transplantation (investigator: J. Shapiro). Protocol design will be similar to that listed above.

Autoimmune diseases

Despite impressive accomplishments in small-animal models, restoration of immune tolerance in the face of an established autoimmune response is an elusive goal. And in contrast to the other diseases within the mission of the ITN, autoimmunity presents additional challenges stemming from limited knowledge of the relevant antigens, complexities related to epitope spreading and multisystem involvement, and few opportunities to intervene early in the course of disease or before onset. Despite these hurdles, autoimmune diseases account for the largest share of proposals to the ITN and of studies selected for implementation. In addition to the trials highlighted below, the ITN is actively seeking novel applications of soluble mediators, such as IL-4, IL-10, transforming growth factor β and anti-IL-10/IL-12, anti-TNF, or anti-IFN- γ antibodies in clinical scenarios not currently a focus of the pharmaceutical industry.

Ongoing ITN-sponsored trials or those in protocol development include the following:

- Anti-CD154 in multiple sclerosis (investigator: L. Kasper). This phase II multisite trial examines the effect of costimulatory blockade on disease progression, as assessed with magnetic resonance imaging. This extends a successful phase I study, as well as promising results in animal models.
- Anti-CD3 in new-onset type 1 diabetes (investigator: K. Herold). This multisite trial will assess the efficacy of a nonactivating humanized monoclonal anti-CD3 in new-onset pediatric and adult type 1 diabetes.
- Anti-CD3 in psoriatic arthritis (investigator: M. Clark). This trial will assess the efficacy of a nonactivating humanized monoclonal anti-CD3 in psoriatic arthritis.
- CTLA4-Ig in multiple sclerosis (investigator: S. Khoury). Building on observations of the importance of the CD80/86-CD28 pathways in autoreactivity and promising results in phase I trials, this study will assess the safety and efficacy of costimulatory blockade in early multiple sclerosis.
- Insulin B-chain in type 1 diabetes (investigator: T. Orban). This trial evaluates the safety and efficacy of insulin B-chain peptides delivered with incomplete Freund's adjuvant to induce antigen-specific tolerance in new-onset type 1 diabetes.

Tolerance assays

In each of the disease areas, the ITN's Tolerance Assay Subgroup supports clinical trials by examining the mechanisms that establish and maintain the tolerant state and developing standardized assays that will guide and inform clinical decisions on maintenance or cessation of immunosuppressive therapies. In addition to the ITN core facilities identified in Table I, the Tolerance Assay Subgroup serves as the ITN liaison for 2 registries-repositories of clinical materials and data derived from spontaneously tolerant individuals who have maintained functioning liver and kidney grafts in the absence of ongoing immunosuppression.

LOOKING FORWARD

The ITN is striving to develop a new paradigm in clinical research. Through an open and inclusive framework for investigator-driven research, a cross-disciplinary emphasis on underlying mechanisms, a strong government and regulatory interface, and mutually beneficial collaborations with industry, the ITN hopes to bring the fundamental advances of the previous decade into the immunology clinic. We encourage all investigators who share these goals to visit the ITN Web site, contact ITN members and staff, and propose projects for ITN support.

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