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FOUND IN TRANSLATION

Taming autoimmunity: Translating antigen-specific approaches to induce immune tolerance

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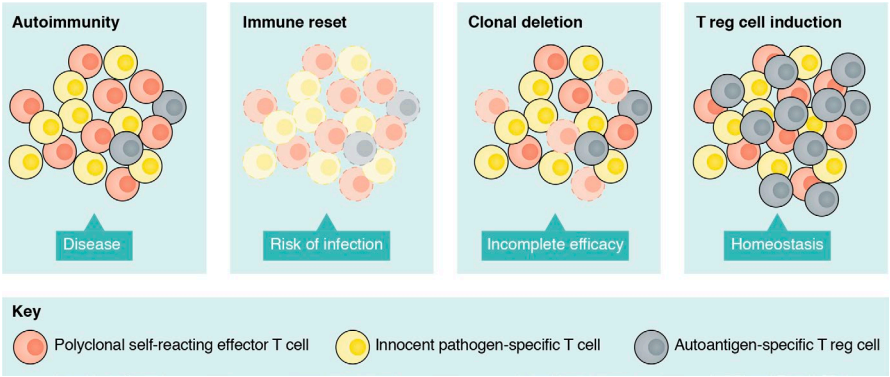
José M. Carballido, Executive Director at Novartis Institutes for BioMedical Research, and Pere Santamaria, Professor of Immunology at the University of Calgary and Founder of Parvus Therapeutics Inc., discuss the opportunities and challenges of translating antigen-specific approaches for autoimmunity with an emphasis on the need for scientific rigor in the preclinical stage.

Immune tolerance is a state of nonresponsiveness (or ignorance) to one or more antigens achieved through a variety of innate or acquired immunological processes. Pharmacologically, induction of tolerance to specific antigens seeks to overcome the need to generate broad immunosuppression as a way to counter pathogenic autoreactivity. Clonal T and B cell deletion in the thymus and bone marrow, respectively, purges the immune system of autoreactive T and B cell specificities recognizing self-antigens with high avidity (Edry and Melamed, 2004; McCaughtry et al., 2007). Clearly, this mechanism does not abrogate self-reactivity completely, since it spares clonotypes with low avidity for thymic antigens or with specificity for peripheral autoantigens that are not expressed in, or ferried to, the thymus and/or bone marrow during T and B cell ontogeny. These autoreactive lymphocytes are normally silenced by mechanisms of peripheral tolerance (Sakaguchi et al., 1995; Rice et al., 2005). However, they can be awakened when their thresholds of activation are reduced (e.g., by disease-predisposing genetic elements and danger signals); and/or when they are suddenly exposed to host autoantigens or cross-reactive antigens derived from infectious organisms in a milieu rich in danger signals. In the case of type 1 diabetes (T1D), an autoimmune disease caused by selective destruction of the insulin-producing β cells of the pancreas, clinical manifestations of autoimmune attack go unnoticed for years, although progressive appearance of autoantibodies in serum helps uncover a smoldering pathological process. Once an autoimmune attack has been initiated, the persistence of antigen and the recruitment of autoreactive T cells targeting other autoantigens (antigen and epitope spreading), together with the low requirements for costimulation characteristic of memory T cells, conspire to fuel a self-sustaining vicious cycle that maintains lifelong disease. At that point, there is little else that can be done other than administer exogenous insulin. Unfortunately, hormonal replacement therapy, which is not available for most other autoimmune diseases, does not tackle the root cause of disease and renders patients subject to the harmful effects of imperfect glucose homeostasis, resulting in a long list of costly chronic complications that diminish the patients' quality of life.



Major mechanisms of immune tolerance. Autoimmunity is mediated by polyclonal self-reacting effector T cells that, following antigen and epitope spreading, largely outnumber the autoantigen-specific T reg cell populations. Tolerance approaches based on immune reset cause complete/temporal immune suppression, as they also eliminate innocent pathogen-specific T cells. Interventions inducing clonal deletion require knowledge of all autoantigen reactivities and therefore have the risk of delivering incomplete efficacy. Induction/expansion of autoantigen-reactive T reg cells (a single disease-relevant epitope specificity is required and sufficient) provides an efficient solution to restore homeostasis.

It is recognized that intervening with immune function represents a major therapeutic hope in T1D and other autoimmune diseases. The problem is that classical immune intervention has relied almost exclusively on broad acting agents, which, although they have shown therapeutic benefits, are not specific for the disease and often increase the risk of infections and malignancies. Besides these scientific arguments, there are other aspects that argue against developing immune therapies that only provide incremental benefits. Both the time and the cost to develop new therapies have been increasing over the last decade. New medicines are now confronted with a situation in which the standard of care offers substantial, yet far from optimal, benefits, and thus the relevance of incremental improvements

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is being called into question. Together, these facts predict that the return on the investment for classical symptomatic therapies will be minimal or even become negative (Stott, 2017). Consequently, research and development in areas with significant medical need might be discouraged. A solution to these problems will be the development of new therapies that specifically dampen the entire polyantigenic autoreactivity of a given autoimmune disease without impairing general immunity. Ideally, such strategies should promote naturally occurring biological pathways, so as to harness mechanisms "discovered" by natural evolution as opposed to blocking them. In other words, there is a need to find therapies that target the root cause of disease and restore immune tolerance.

Induction of immune tolerance could be attempted using a "reset" approach, such as by eliminating all the mature hematopoietic cells followed by immune reconstitution with autologous hematopoietic stem cell precursors. The feasibility of this option has been tested in multiple sclerosis and myasthenia gravis patients (Atkins et al., 2016; Bryant et al., 2016) with promising results. Unfortunately, this approach is associated with unacceptable mortality due to the stringent conditioning required to ensure complete elimination of the host immune repertoire.

A far less aggressive and more amenable choice to promote immune tolerance involves targeting the existing peripheral effector and/or memory autoreactive T cell compartments using antigen-based approaches. These interventions aim to achieve two alternative, albeit not mutually exclusive, general outcomes: (i) clonal inactivation (anergy) and deletion of antigen-specific effector T cells; and/or (ii) de novo generation of inducible regulatory T (T reg) cell types. The therapeutic potential of each of these two outcomes is fundamentally different. Deletional approaches might be effective in situations that are driven/sustained by monospecific and well-defined autoreactive T and/or B cell specificities and display minimal antigen and/or epitope spreading. Examples of these are anti-drug immune responses triggered by repeated administration of exogenous antigenic material, such as proteins used to treat inflammatory, metabolic, or genetic disorders (e.g., uricase, factor VIII, etc.), and therapeutic antibodies or gene therapy vectors. Additional examples include autoimmune diseases like pemphigus vulgaris or celiac disease, in which the pathology is caused by autoantibodies or T cells targeting a single or few autoantigens (i.e., desmoglein 3 in pemphigus or gliadin epitopes in celiac disease). In such mono- or pauciantigen-driven conditions, specific deletion of cognate T cells might be effective.

The use of deletional approaches to blunt autoimmune diseases driven by cellularly and antigenically complex immune responses is much less appealing for two main reasons. First, we do not fully comprehend the sequences of events that cause and sustain diseases like T1D, multiple sclerosis, rheumatoid arthritis, liver autoimmune diseases, or inflammatory bowel disease, just to name a few. In addition, we have a woefully incomplete knowledge of the underlying antigenic repertoires that sustain pathology. Accordingly, it is hard to imagine how deletion of a fraction of the autoantigenic T and/or B cell repertoires in these disorders, should that be pharmacologically possible, might be able to blunt disease progression. Even if such interventions could be delivered early in the disease process before antigen and epitope spreading takes hold, there are no guarantees that once T cells displaying such cognate autoreactivity are eliminated, they will not be replaced by T cells recognizing subdominant epitopes (i.e., such as those encoding cryptic, posttranslationally modified, or hybrid antigenic epitopes, as described in T1D; James et al., 2018).

It thus stands to reason that approaches capable of eliciting immunoregulatory processes in which therapeutic potency is independent of the degree of autoantigenic diversity sustaining disease would be more appealing. Pharmacologic expansion of autoantigen-specific T reg cells and local neutralization of antigen presentation, either by inactivating or killing APCs carrying the polyclonal autoantigen load, meets these criteria. Locally, at the site of inflammation or in the draining lymph nodes, autoantigen-specific T reg cells will be activated upon recognition of their target peptide-MHC (pMHC) molecules on the surface of professional, autoantigen-loaded APCs, and there they will dampen the recruitment, activation, and retention of all disease-relevant effector T cells and therefore blunt disease progression.

Several antigen-specific tolerogenic approaches are currently under development (Table 1). This opinion article is not intended to review the strengths and weaknesses of these different approaches (see Serra and Santamaria [2019] for a detailed review), but rather to define the common ground required to increase the likelihood of success. The scientific rigor of the preclinical work supporting these various strategies, as described in the literature, is very heterogeneous, and therefore there is a high risk that unsuccessful trials based on questionable interpretation of incomplete scientific data discourage future attempts to achieve the fundamental mission of realizing immune tolerance. This helps no one, least of all the patients in need.

To avoid this situation, it is important to learn from the shortcomings of previous efforts. It is of fundamental importance to have a detailed understanding of the mechanism of action (MoA) of the therapeutic principle. Scientific advances leading to breakthrough therapeutic approaches are often triggered by serendipitous observations made while pursuing curiosity-driven research, but clinical translation of these discoveries requires a thoughtful and methodical experimental follow-up. Testing the robustness of the MoA in multiple in vitro and in vivo models is of paramount importance. Ideally, the preclinical autoimmune disease models should be driven by spontaneous processes and/or be induced using a variety of antigens and in multiple genetic backgrounds, so as to mimic as best as possible the overwhelmingly complex polyclonal immune responses characteristic of human autoimmune diseases. Cell transfer experiments using T cell receptor transgenic mouse donors, for example, can provide valuable mechanistic information but are inadequate to demonstrate therapeutic efficacy or MoA. The drug candidates might work prophylactically when administered before manifestations of overt disease, but this cannot be taken as a predictor of therapeutic utility in patients with overt disease. In addition, treatment should provide durable benefits without the need for short-interval repetitions of the treatment. Multiple redundant readouts of preclinical therapeutic activity should be carefully evaluated. For approaches claiming antigen-specific T reg cell induction or expansion, demonstration of the T reg cells' specificity and phe-

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Table 1. **Ongoing approaches to induce immune tolerance**

Type of approach	Modality	Institutions supporting the concept
Clonal deletion using pre-apoptotic cells	With autologous peripheral blood mononuclear cells; in vitro coupled to a cocktail of autoantigen-derived peptides prior to cell transfer	Cellerys
	With autologous RBCs; in vitro coupled or loaded with autoantigens/autoantigen-peptides	Rubius Therapeutics, SQZ Biotechnologies
	With autologous RBCs; in vivo targeted with RBC-binding molecules fused to autoantigens/autoantigen-peptides	Anokion/Celgene, Kanyos (Anokion/Astellas)
Therapeutic immunization	With peptide or whole autoantigen proteins, alone or as cocktails, with or without adjuvants	Apitope, Diamyd Medical, Immusant, Orban Biotech, UCB Pharma
	With DNA vaccines	Tolerion
	With autoantigenic peptides containing thioredoxin motifs	Imcyse
Cell-based approaches	Transferring autologous dendritic cells differentiated in vitro using cytokines, vitamin D3, dexamethasone, or genetically engineered to downregulate costimulatory molecules	Baylor Research Institute, Diavacs, Leiden University
	Transferring in vitro inactivated autologous autoantigen-specific T cells to expose ergotypic antigens	Opexa Therapeutics
	Transferring autologous regulatory chimeric antigen receptor-T (CAR–T reg) cells	Txcell/Sangamo
	Administering engineered bacteria expressing host autoantigens together with host immune modulators	ActoBio/Intrexon, Allero Therapeutics
Engineered nanomedicines	Delivering autoantigenic peptides/proteins, alone or in combination with immunomodulatory agents, to APCs using nanoparticle vehicles	AntolRx/Pfizer, Cour Pharmaceuticals, Dendright/Janssen Biotech, Midatech Pharma, Regimmune, Selecta Biosciences, Toleranzia, Topas Therapeutics, Toralgen
	Directly targeting autoantigen-specific T cells with pMHC proteins coated onto nanoparticles, to reprogram and expand cognate T reg cells	Parvus Therapeutics/Novartis

notypic and functional hallmarks, both in vitro and in vivo, is also critical (e.g., pMHC tetramer staining, ability to transfer disease suppression into spontaneous disease models, etc.); claims based on small elevations in total (polyclonal) T reg cell levels or on the ability of systemic T reg cell depletion (i.e., with depleting mAbs) to abrogate therapeutic activity can be misleading in the absence of these data. Antigen-specific tolerogenic strategies should be devoid of off-target side effects and should not exacerbate disease or promote general immune suppression. Although laborious, incorporation of humanized mouse models during preclinical evaluation may help support the viability of the mouse-to-human translational leap. We recognize that some of the above suggestions may not be applicable to all therapeutic modalities or disease indications (i.e., those without informative animals models). Nevertheless, efforts to address them would help "raise the bar" and thus increase the odds of success for everyone involved in the development of immune tolerance.

From the manufacturing point of view, several key aspects need to be considered. The drug should be scalable to allow formal preclinical and clinical testing and subsequent commercialization within a reasonable cost range to enable broad and fast access to all patients in need. Many of the approaches mentioned above will need to be customized to specific patient populations. Precision therapies requiring a certain degree of personalization would be perfectly viable if they do not require the development of complex and costly individual treatments or the development of many different products per disease indication.

The clinical testing of immune tolerance therapeutics should also be carefully designed. There will be scientific, regulatory, and ethical aspects that may suggest testing the therapeutic principle in a healthy population first or, to the contrary, support moving directly into the patient population. In the latter case, special attention should be given to minimizing the risk of exacerbating autoimmunity while seeking a proof of mechanism or proof of concept. Of note, standard protocols involving the initial use of single ascending doses do not necessarily apply to immunotherapies that aim to induce tolerance, where pharmacodynamic and therapeutic activity are a function of dosing frequency and number. In this regard, it is extremely important to develop biomarker assays that can inform both target engagement and pharmacodynamic effects. These biomarkers will not only help shorten the time required to declare therapeutic success, but will also inform the care provider on the need for re-treatment to maintain long-term tolerance. Finally, data collection could also benefit from the use of digital health "wearables" capable of continuous health monitoring over the entire trial period.

Immune tolerance represents a transformative concept with significant game-changing potential. It is envisioned that patients with recent autoimmune disease onset will experience fast benefits reverting to the homeostatic steady state. Patients with long-lasting disease could also benefit from these immune tolerance therapies either alone or in combination with tissue repair/regenerative approaches, since dampening the inflammatory pressure on the target organs may be sufficient to restore tissue functionality. Finally, screening patients at risk for early signs of disease, such as the presence of disease-associated

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autoantibodies, might support prophylactic interventions. Overall, there is a high likelihood that, in the near future, precision immune tolerance therapeutics will be able to tame today's lifelong autoimmune diseases into manageable acute events.

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J.M. Carballido is an employee of Novartis Pharma AG and is involved in drug development work. P. Santamaria is scientific founder of Parvus Therapeutics Inc. and has a financial interest in the company.

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