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Treating Human Autoimmunity: Current Practice and Future Prospects

Michael D. Rosenblum^{1,*}, Iris K. Gratz², Jonathan S. Paw¹, and Abul K. Abbas^{2,*}

¹Department of Dermatology, University of California, San Francisco, San Francisco, CA 94115, USA

²Department of Pathology, University of California, San Francisco, San Francisco, CA 94143, USA

Abstract

Autoimmune diseases are caused by immune cells attacking the host tissues they are supposed to protect. Recent advances suggest that maintaining a balance of effector and regulatory immune function is critical for avoiding autoimmunity. New therapies, including costimulation blockade, regulatory T cell therapy, antigen-specific immunotherapy, and manipulating the interleukin-2 pathway, attempt to restore this balance. This review discusses these advances as well as the challenges that must be overcome to target these therapies to patients suffering from autoimmune disease while avoiding the pitfalls of general immunosuppression.

INTRODUCTION

Autoimmune disease contributes substantially to morbidity, mortality, and health care cost each year. More than 50 million Americans are currently living with an autoimmune disease, and new cases are rising at an alarming rate (1). Autoimmunity is the highest cause of morbidity in women in the United States and is one of the top 10 causes of death in women under the age of 65 (2). Autoimmune diseases are frequently chronic illnesses, and it is estimated that more than 100 billion health care dollars are spent each year in the management of autoimmune patients, which places autoimmunity among the most costly diseases to diagnose and treat.

Autoimmune diseases can be either systemic or tissue-specific in nature; however, all forms of autoimmunity are thought to result from a disruption of balance within the immune system. The normal immune system is designed to recognize and react to a multitude of foreign pathogens while remaining unresponsive to host tissues (such as self-antigens). This ability to live with—or tolerate—self is known as immunological tolerance. Although lymphocytes specific for self-antigens are constantly being generated in the thymus (termed T lymphocytes or T cells), many of these cells are eliminated before they complete their maturation. However, this process is imperfect. Normal healthy individuals have circulating T cells that are capable of mounting pathogenic immune responses directed at self-antigens

*To whom correspondence should be addressed. rosenblummd@derm.ucsf.edu (M.D.R.); Abul.Abbas@ucsf.edu (A.K.A.).

(3). Nevertheless, most people do not develop autoimmune disease. Instead, in healthy individuals, the pathogenicity of these self-reactive cells is counterbalanced by regulatory mechanisms that are constantly at work suppressing potentially damaging responses, thus maintaining tolerance to self.

These observations have led to a paradigm shift in the study of autoimmunity. Autoimmune disease is no longer thought to be triggered solely by signals that lead to the aberrant activation of self-reactive cells, but instead may primarily result from a defect in the ability to control these cells. Using the crude analogy of an automobile, auto-immunity does not simply result from stepping on the gas pedal, but also requires letting up on the brake. Elucidating the mechanisms that naturally suppress self-reactivity is central to understanding the pathogenesis of autoimmunity, and exploiting these pathways for therapeutic benefit may provide exciting and novel targeted approaches to treat autoimmune and inflammatory disease.

TREATING AUTOIMMUNITY

Traditional therapies for autoimmune disease have relied on immunosuppressive medications that globally dampen immune responses. These agents are highly effective for many patients and thus remain the current “gold standard” of care. However, long-term treatments with high doses are often needed to maintain disease control, leaving the patient susceptible to life-threatening opportunistic infections and long-term risk of malignancy. In addition, the benefits of many of these drugs are counterbalanced by toxicity and serious side effect profiles. Thus, there has been a push for the development of more specific strategies that lower the risk of systemic immune suppression and improve tolerability. The optimal therapy for autoimmunity would be one that achieves four main goals: (i) specifically targets the pathogenic cells and leaves the remainder of the immune system functioning normally; (ii) reestablishes immune tolerance that is stable over time, such that continuous or long-term therapy is not needed; (iii) has low toxicity and few side effects; and (iv) is overall cost-effective when compared to alternative approaches.

The new wave of treatments for autoimmune disease strives to achieve these goals. Mechanistically, these approaches either focus on inhibiting the activation of pathogenic cells or are aimed at augmenting the pathways that naturally suppress these cells. Herein, we discuss the major advantages and limitations to some of the newest approaches to treat T cell-mediated autoimmunity, with specific focus on strategies to overcome current roadblocks.

COSTIMULATORY BLOCKADE

Activation of T cells requires two main signals (Fig. 1). The first signal is antigen recognition through the T cell receptor (TCR), and the second signal is a costimulatory signal provided by the cell presenting the antigen [antigen-presenting cell (APC)]. Both signals are required to achieve full T cell activation. If the first signal is received without the second, T cells become suboptimally stimulated, resulting in a state of unresponsiveness or anergy (4). In the context of infection with a foreign pathogen, various inflammatory signals increase expression of costimulatory molecules on APCs, leading to full T cell activation.

Autoreactive T cells are normally presented self-antigens in the absence of inflammation, receiving a TCR signal (signal 1) without costimulation (signal 2), which effectively renders these cells functionally anergic. However, in the context of autoimmunity (and associated inflammation), self-reactive T cells may receive both signal 1 and signal 2, resulting in full activation. These cells are then capable of mounting pathogenic immune responses in tissues that express their cognate antigen.

Because costimulation is required for T cell activation, blocking costimulatory pathways is an attractive potential treatment for auto-immune disease. In this approach, costimulatory signals are specifically inhibited in an attempt to render self-reactive T cells anergic and attenuate the overall autoimmune response. The greatest success to date of this approach is cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)–immunoglobulin (Ig), which directly prevents costimulation mediated by CD28 [Fig. 1; (5)]. CD28 was the first costimulatory receptor identified on T cells, and signaling through this receptor initiates a potent T cell activation cascade resulting in proliferation and functional differentiation. The chimeric CTLA-4–Ig protein combines the extracellular domain of CTLA-4 with human IgG1. It is a soluble receptor fusion protein that binds two potent costimulatory ligands on APCs, B7-1 and B7-2 (also called CD80 and CD86). In doing so, CTLA-4–Ig inhibits the ability of these molecules to bind to CD28 (their receptor on T cells). Treatment with CTLA-4–Ig (abatacept) has been shown to be effective in both rheumatoid arthritis (RA) and psoriatic arthritis and has recently shown promise in treating type 1 diabetes (6–8). In one study, 112 patients with recent-onset type 1 diabetes were treated with abatacept or placebo over a 2-year period. Patients treated with abatacept showed a slower reduction in pancreatic β cell function. However, despite monthly administration of the drug over 24 months, the beneficial effects were only observed within the first 6 months of therapy (8).

Despite efficacy in RA, psoriatic arthritis, and type 1 diabetes, costimulatory blockade has been less promising for the treatment of systemic lupus erythematosus (SLE), multiple sclerosis, and inflammatory bowel disease (9–11). Perhaps, this is because this form of treatment blocks activation of naïve T cells, and thus, it may have less of an effect on previously activated T cells or long-lived memory T cells. Once self-reactive T cells have been activated, blocking costimulatory molecules may be unable to suppress the pathogenicity of these cells. Consistently, costimulation blockade works much better in preventing disease than in treating active disease in preclinical animal models of autoimmunity (12). This limitation may also explain why abatacept preferentially works during the early phase of type 1 diabetes, because new T cell activation most likely contributes to the pathogenesis only early in the disease process (8). On the basis of these results, prevention trials for type 1 diabetes are being planned. Because people with prediabetes can be identified before they develop clinical manifestations, prevention in this context may be feasible.

Another limitation of costimulatory blockade is the need for continuous treatments. This approach has not been shown to induce stable self-tolerance with short courses of therapy. In addition, blocking costimulatory signals is not specific for autoreactive T cells. Thus, this approach has the potential to inhibit T cell responses to foreign pathogens, leaving patients susceptible to potentially life-threatening infections.

REGULATORY T CELL THERAPY

Although multiple mechanisms exist to control immune responses directed at self-antigens, one of the most important of these relies on a unique population of T lymphocytes called regulatory T cells (T_{regs}), whose discovery in the late 1990s has been a milestone in immunology. T_{regs} are a subset of $CD4^{+}$ T cells that express high levels of the interleukin-2 (IL-2) receptor α chain CD25 and the transcription factor Foxp3 [reviewed in (13)]. T_{regs} unequivocally have been shown to suppress pathogenic immune responses directed at self-antigens; both mice and humans with nonfunctional T_{regs} develop florid auto-immunity. *Foxp3*-deficient mice develop de novo autoimmune gastritis, thyroiditis, diabetes, dermatitis, and inflammatory bowel disease, and die around 3 to 4 weeks of age (14). People with mutations in the *Foxp3* gene have a similar phenotype. They develop autoimmune enteropathies, endocrinopathies, and failure to thrive, all of which are manifested within the first few months of life (15). Without a bone marrow transplant, these patients usually die during childhood. The role of T_{regs} in preventing more common autoimmune diseases is a subject of intense current interest that has led to an emerging paradigm: The outcome of all immune responses, including those directed at self-antigens, is determined by the ratio of functional effector (or conventional) T cells to T_{regs} .

Because T_{regs} have a potent and obligatory role in suppressing auto-immunity, they have emerged as the quintessential cell population to exploit for therapeutic benefit. The idea behind this approach is simple: isolate these cells, activate and expand them ex vivo to high numbers, and adoptively transfer them to patients with autoimmune disease in an attempt to suppress (and potentially cure) the ongoing autoimmune response (Fig. 2). However, the implementation of this approach has been fraught with pitfalls and impediments. Although this idea was first proposed more than 15 years ago, clinical trials exploring the safety and potential efficacy of adoptively transferred T_{regs} in treating human autoimmunity are only now being realized, mostly because of a lack of knowledge about the fundamental biology of these cells in human beings.

The ideal population of T_{regs} for adoptive transfer consists of highly pure antigen-specific T_{regs} that are stable and potent suppressors capable of specifically regulating autoimmunity in the affected organ(s) without causing global immunosuppression. The first obstacle in obtaining such a population is the inability to isolate highly pure T_{regs} . Both conventional $Foxp3^{+}$ T_{regs} and unconventional $Foxp3^{-}$ T_{regs} [such as IL-10-producing type 1 T_{regs} , or Tr1 cells; reviewed in (16)] lack specific cell surface markers that allow for their purification and separation from potentially pathogenic T cells. The best cell surface marker to date is CD25; however, cell purification-based techniques using this marker typically yield between 60 and 90% $Foxp3^{+}$ cells (17). Foxp3 itself cannot be used because it is an intracellular protein, and permeabilization of cells to identify Foxp3 expression renders them nonviable. Newer approaches have attempted to use other cell surface markers, including CD127, CD45RA, CD121, and latency-associated peptide (18, 19); none of which have proven to yield universally accepted T_{reg} purities. The question of what is an “acceptable” T_{reg} purity remains controversial as well, as even a small percentage of contaminating T cells can theoretically exacerbate autoimmune disease after activation and expansion ex vivo.

Because Foxp3-expressing T_{regs} comprise roughly 2% of CD4⁺ T cells in human peripheral blood, these cells need to be expanded to high numbers to be useful in adoptive transfer approaches. A lack of knowledge of the molecular pathways that specifically activate T_{regs} or promote T_{reg} differentiation (and how this differs from the activation and differentiation of conventional T cells) has resulted in most ex vivo T_{reg} expansion protocols using nonspecific T cell activation methods, such as TCR stimulation and exogenous IL-2 (a pan-T cell growth factor) (20–22). Because these conditions are not specific for T_{regs}, contaminating T cells (that are potentially pathogenic) are also expanded. However, the addition of the antiproliferative compound rapamycin to T_{reg} cultures favors the growth of CD4⁺CD25⁺Foxp3⁺ T_{regs} over CD4⁺CD25[−] T cells and also supports the suppressive function of cultured T_{regs} (22–24). Another limitation to ex vivo T_{reg} expansion is the culture time required to obtain adequate numbers of these cells, which is exacerbated by the potential of T_{regs} to lose their suppressive capacity with time in culture (25).

Most methods of testing human T_{reg} function rely on in vitro suppression assays, and these methods of ascertaining T_{reg} function may not accurately reflect the suppressive capacity of T_{regs} in vivo. Testing their ability to suppress autoimmune reactions in human beings would clearly be the best assay to ascertain the function of in vitro-expanded T_{regs}; however, because there are clear ethical and practical concerns with this approach, assaying the suppressive capacity of these cells in humanized mouse models may suffice.

Questions regarding the functionality of these cells are further complicated because in certain contexts, the suppressive function of T_{regs} may not be stable over time. Cells that once expressed Foxp3 can transition to nonregulatory Foxp3[−] pathogenic T cells. Indeed, in animal models, cells that once expressed Foxp3 have been shown to convert to pathogenic effector cells capable of inducing type 1 diabetes (26). In human beings, Foxp3-expressing cells are capable of producing both T helper 17 (T_H17) and T_H1 cytokines upon ex vivo activation (27, 28)—the latter of which are increased in patients with untreated relapsing-remitting multiple sclerosis (29). Thus, even if a relatively pure population of T_{regs} could be obtained and selectively expanded, it is unclear whether such cells would stably retain their immunoregulatory capacity and suppress autoimmune reactions upon adoptive transfer to patients.

Despite current limitations in adoptive T_{reg} therapy, a disease setting where this approach is proving to be successful is acute graft-versus-host disease (aGVHD). aGVHD is a “man-made” autoimmune-like disease that can result after bone marrow transplantation. Lymphocytes are administered in the bone marrow or stem cell inoculum that recognize and react against host tissue antigens—primarily host human leukocyte antigen (HLA) molecules. In this disease setting, both host and donor tissue are accessible before cell administration, which allows for the ex vivo expansion and activation of host-reactive T_{regs} before adoptive transfer (30). Because the onset of disease is highly controlled (for example, several days to weeks after transfer of host-reactive cells), T_{regs} can be administered before the initiation of pathogenic anti-host responses, effectively giving these cells a “head start” in preventing and/or suppressing the disease process.

Taking advantage of many of these characteristics of aGVHD, Di Ianni and colleagues recently reported a series of 28 patients that were given donor T_{regs} before adoptive transfer of hematopoietic stem cells and pathogenic T cells (CD4⁺CD25⁻ cells) in HLA-haploidentical hematopoietic stem cell transplantation (HSCT) for hematologic malignancy (31). In this setting, about half of the HLA molecules were mismatched between donor T cells and host tissues, which predisposes recipients to a high incidence of severe aGVHD. This type of HSCT traditionally requires high doses of immunosuppression in the post-transplant period and frequently results in lethal aGVHD (32). Only 2 of 26 patients in this study developed moderate-to-severe aGVHD, and no patients received posttransplant immunosuppression. In addition, in the setting of umbilical cord blood (UCB) transplantation, Brunstein and colleagues have recently shown that adoptive transfer of polyclonal T_{regs} was well-tolerated, safe, and resulted in a reduced incidence of severe aGVHD when compared to historical controls (33). Together, these studies show, for the first time in human beings, that adoptive transfer of T_{regs} can prevent severe aGVHD and possibly replace the need for high doses of immunosuppressive medications in the posttransplant period. After the pioneering work done in GVHD, investigators are now enrolling patients in clinical trials to study the safety and efficacy of adoptive T_{reg} therapy in other auto-immune diseases, including type 1 diabetes (Table 1).

RESTORING TOLERANCE WITH ANTIGEN ADMINISTRATION

If self-antigens that are targeted in various autoimmune diseases are identified, it should be possible to administer these antigens to patients in ways that inhibit rather than promote autoimmune responses. The proof of principle for this approach has been in clinical practice for several years in the context of allergy. Allergen-specific immunotherapy (allergen-SIT) is an effective therapy to suppress immuno-reactivity to allergens and represents the only curative and specific modality to treat allergic disease [reviewed in (34)]. In this approach, specific antigens to which the patient is allergic—have uncontrolled T_H2-type immune responses—are repeatedly administered (either cutaneously or orally) in increasing doses, resulting in robust suppression of the allergic reaction that is stable over time. This strategy is currently being successfully used for the systemic desensitization of multiple environmental and drug allergens. It also works for other antigens in nonallergic contexts. Perhaps, the best “real-world” example of this is the nonallergic beekeeper. Bee venom contains several different foreign antigens that are directly inoculated into the skin with a single bee sting. During the beekeeping season, a beekeeper is stung multiple times per week. With successive weeks into the season (and repeated stings), both cutaneous and systemic anti-venom immune responses decline to almost undetectable levels, and these individuals become completely desensitized to bee venom antigens (35), in many cases resulting in life-long tolerance (36).

Multiple mechanisms have been suggested for desensitization to both allergens and bee venom antigens, with increasing evidence supporting the preferential augmentation of antigen-specific T_{regs}. Studies have shown that antigen-SIT effectively increases the number of antigen-specific T_{regs} relative to antigen-specific pathogenic T cells (37–39). In mouse model of inducible self-antigen expression in the skin, persistent antigen exposure resulted in a marked increase in the T_{reg}/pathogenic T cell ratio because of a preferential reduction in

pathogenic T cell numbers and relative preservation of T_{regs} in the presence of self-antigen. In addition, persistent expression of self-antigen in tissues led to T_{reg} activation, proliferation, and heightened suppressive capacity, which suggests that exposure to self-antigen not only increases the relative number of T_{regs} but may also enhance T_{reg} function (40).

This therapeutic approach might be clinically applicable to self-antigens in the context of autoimmunity. Groups are now testing whether repeated exposure to self-antigen leads to a preferential induction of antigen-specific T_{regs} that are able to suppress an ongoing autoimmune response. In the setting of SLE, three doses of self-peptide were delivered subcutaneously to patients with active disease. This treatment resulted in a significant decrease in autoantibody production and an improvement in clinical disease severity (41). In the setting of auto-immune diabetes, antigen-SIT trials with insulin as the tolerizing antigen have been disappointing (42). However, clinical trials using DiaPep277, a self-antigen targeted in type 1 diabetes, have resulted in preservation of insulin production in patients with established disease and a trend toward increased pancreatic β cell function in patients with recent-onset disease (43, 44). Similar trials are under way in multiple sclerosis, in which myelin peptides will be administered in an attempt to attenuate disease and restore immune tolerance.

A variation of this approach is to modify self-peptides to make them more potent at inducing tolerance. Injection of self-peptides chemically coupled to spleen cells can successfully induce self-tolerance in mice (45). Recently, it has been shown that this method of tolerance induction is linked to the increased expression of immunoregulatory proteins on APCs and the activation of antigen-specific T_{regs} (46). Together, these studies provide a strong rationale for antigen-SIT in treating auto-immune disease.

The great advantage of antigen-induced tolerance over other therapeutic modalities is that this approach blocks only the harmful response and does not cause broad immunosuppression, with its attendant risk of infection. However, despite excellent results in the setting of allergy, sound preclinical studies, and promising (but limited) clinical data, antigen-SIT has several limitations that must be overcome before its true efficacy can be realized in the setting of autoimmunity. Perhaps the most important caveat of this approach is our lack of knowledge of the dominant antigens that drive most autoimmune diseases. Moreover, these antigens may change over time and can differ between patients with similar clinical disease. In addition, on the basis of what we have learned in mouse models and in the clinical setting of allergy, antigen would need to be repeatedly inoculated to maximize T_{reg} activation and accumulation over pathogenic T cells. Finally, administration of the antigens that cause autoimmunity always carries the risk of exacerbating the autoimmune response; although this remains a concern, it has not occurred in most of the trials done so far.

MANIPULATING THE IL-2 PATHWAY

IL-2 is a T cell growth factor with opposing roles in the immune system. On the one hand, IL-2 can enhance immune responses by promoting the proliferation and generation of

effector T cells. On the other hand, IL-2 can indirectly suppress immune response by promoting the survival and function of T_{regs} [reviewed in (47)]. Various groups have attempted to exploit the IL-2 pathway in an attempt to preferentially enhance T_{reg} numbers and/or function to treat autoimmunity through the use of recombinant IL-2 bound to anti-IL-2 antibody [rIL-2-IL-2 monoclonal antibody (mAb) complexes]. Cytokine/anti-cytokine antibody immune complexes have been shown to preferentially stimulate specific T cell subsets (48). In the context of IL-2, low doses of rIL-2-IL-2 mAb complexes preferentially stimulate T_{regs} (Fig. 2) (49). T_{regs} expanded using this approach are able to suppress autoimmune reactions in mouse models of hemophilia (50), myasthenia gravis (51), experimental autoimmune encephalomyelitis (52), and auto-immune diabetes (53).

Although promising in preclinical mouse models, studies validating this approach in human autoimmune diseases are lacking. However, a similar approach with IL-2 in noncomplex form (in the absence of anti-IL-2 antibody) has been attempted. Recently, administration of low-dose recombinant IL-2 in patients with steroid-resistant chronic GVHD resulted in T_{reg} expansion and clinical benefit, although the number of patients treated was small (54). Because this treatment is not antigen-specific, global immune suppression by way of pan-T_{reg} activation remains a concern. In addition, because IL-2 signaling can stimulate conventional T cells (non-T_{regs}) as well, this approach has the potential to result in some degree of activation of pathogenic T cells, which could exacerbate disease. Nevertheless, promising preclinical data in mouse models suggest that rIL-2/IL-2 mAb complexes may be effective in treating human autoimmune disease in specific settings.

OVERCOMING CURRENT LIMITATIONS

Many of the treatment modalities currently being investigated do not fulfill all four criteria that constitute the “perfect” therapy for auto-immune disease. However, we are getting close. Lack of specificity and an insufficient understanding of the biology of T_{regs} remain the two largest obstacles.

Costimulatory blockade, adoptive transfer of polyclonal T_{regs}, and manipulation of the IL-2 pathway all lack specificity and may attenuate most or all immune responses. These approaches confer a higher risk of systemic immune suppression, which is a major limitation of traditional treatments. Newer therapies may adopt a more targeted approach and will thus require less overall therapeutic intervention when compared to traditional therapies (for example, fewer doses over a shorter period of time may be needed to achieve durable disease control). However, only time will tell if these newer modalities will carry a lower risk of severe complications while maintaining efficacy.

In contrast to other approaches, antigen-SIT is highly specific. In the context of allergy, this treatment confers antigen-specific immune tolerance with virtually no compromise in the ability to generate immune responses to other antigens. However, for this intervention to be effective, the antigen(s) driving the autoimmune response must be known. Thus, discovery of the major antigens targeted in human auto-immune diseases will greatly enhance our ability to reestablish antigen-specific immune tolerance in both antigen-SIT and all other current tolerance-promoting strategies. Recent advances in high-throughput screening

approaches with self-protein libraries and autologous T cells from autoimmune patients will undoubtedly enhance our ability to do so (55). It is imperative that sufficient research initiative and financial resources are allocated to these lines of investigation.

Because many new and exciting treatments for autoimmune disease attempt to exploit T_{regs} to reestablish and maintain immune homeostasis, it is essential that we learn more about the fundamental biology of these cells, especially in human beings. Selection markers that better discern these cells from their pathogenic counterparts are needed. In addition, a better understanding of the factors that preferentially activate T_{regs} over conventional T cells is imperative. These advances will allow for the preferential augmentation of T_{regs} both in vivo and ex vivo. In addition, several experimental models of auto-immunity have shown that T_{regs} mediate suppression in the tissues where the autoimmune response is occurring (40, 56, 57). To do so, they acquire specific chemokine receptor profiles that enable them to migrate to the target tissue. Understanding how T_{regs} acquire and/or maintain tissue-specific homing properties will undoubtedly optimize the in vivo function of these cells as well as improve targeted immune regulation resulting in fewer systemic side effects.

WHERE DO WE GO FROM HERE?

One of the primary objectives in autoimmune research should be specificity. Aside from discovering the specific antigens that drive human autoimmune disease, we must elucidate mechanisms that specifically augment T_{reg} numbers and suppressive capacity both ex vivo and in vivo. This may be accomplished by discovering ways to genetically modify IL-2 so that it preferentially binds to T_{regs}, or through the discovery of costimulatory and/or chemokine pathways that are specific to T_{regs}. This work can be done with existing mouse models but should be expeditiously translated to test if the same principles hold true in human beings.

In addition, combination therapies should be explored. Enhancing the therapeutic efficacy and side effect profiles of current approaches will most likely encompass some combination of antigen specificity and T_{reg} augmentation. For example, blocking costimulation of pathogenic T cells in combination with preferentially activating antigen-specific T_{regs} may result in long-lived immune tolerance. Antigen-SIT in combination with approaches that preferentially augment T_{regs} (such as rIL-2/IL-2 mAb complexes) may be more effective than antigen-SIT alone.

Different treatment approaches will most likely be more or less effective in specific disease settings. Antigen-SIT might be most effective in the treatment of tissue-specific autoimmune diseases, such as autoimmunity of the skin. Unlike autoimmune diseases affecting other organs, many of the self-antigens driving the most common types of cutaneous autoimmunity are known (58). In addition, the skin is a highly accessible tissue and local expression of antigen results in skin-specific T_{reg} accumulation, directly recruiting these cells to their site of function (the target tissue) and limiting their capacity to cause systemic immune suppression (40). In contrast, systemic autoimmune diseases like RA and SLE may respond best to treatments that induce systemic tolerance, such as costimulatory blockade with adoptive transfer of antigen-specific T_{regs}.

In a relatively short amount of time, we have learned an enormous amount about how the normal immune system suppresses self-reactivity and how defects in the ability to do so result in autoimmune disease. Many of the landmark studies that have elucidated novel tolerance-inducing strategies have been done in mouse model systems. Now, we are on the cusp of translating these approaches to treat human disease. Moving forward, a better understanding of the human immune system will undoubtedly help us overcome inherent limitations to current therapies.

In addition to well-controlled and ethically sound clinical trials, this work will also most likely rely on preclinical humanized mouse models. Humanized mice are genetically modified to be selectively immunodeficient, which enables them to accept and stably engraft human tissue. These systems allow for the study of human immune responses in mice and are proving to be an extremely valuable tool for modeling human autoimmunity (59).

In conclusion, there is cause for optimism that the days of non-specific immunosuppressive medications with limited efficacy, high toxicity, and life-threatening side effects might end as the new generation of immunotherapy pushes forward. Certainly, we are making strides toward achieving the perfect treatment for autoimmunity. Thus, it may no longer be prudent to ask if we will ever get there, but instead, it may now be just a question of when.

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Fig. 1.

Costimulatory blockade as a method to treat autoimmunity. T cells require two signals to become fully activated. The first signal (“signal 1”) is provided through the TCR upon recognition and binding of specific antigen presented in the context by MHC (major histocompatibility complex) molecules on APCs. The second signal (“signal 2”) is a costimulatory signal provided by engagement of costimulatory ligands expressed on APCs with costimulatory receptors expressed on T cells. If T cells receive signal 1 without signal 2, they fail to be fully activated and are rendered functionally anergic. CD80 (B7-1) and CD86 (B7-2) binding to CD28 provides a robust costimulatory signal to T cells. Abatacept and belatacept are chimeric fusion proteins consisting of the extracellular domain of CTLA-4 and human IgG1. They bind to CD80 and CD86 with high affinity, preventing binding of these costimulatory ligands to CD28. Through blocking this potent costimulatory pathway, these reagents inhibit the activation of auto-reactive T cells in patients with autoimmunity.

Fig. 2.

Augmentation of T_{reg} s to treat human autoimmunity. Both in vivo and ex vivo approaches have been used to enhance the relative numbers of T_{reg} s to treat autoimmune disease. Adoptive T_{reg} therapy is a method by which polyclonal T_{reg} s are purified from peripheral blood or cord blood via fluorescence-activated cell sorting using cell surface markers preferentially expressed on T_{reg} s. Purified cells are expanded ex vivo by culturing in the presence of TCR stimulation (with or without costimulation) in combination with exogenous IL-2 and, in some cases, rapamycin (which preferentially inhibits conventional T cell proliferation in vitro). Expanded cells are then adoptively transferred to the patient. The process of self-antigen-specific tolerance is aimed at enhancing the T_{reg} -to-pathogenic T cell (T_{eff}) ratio in vivo. In this approach, self-peptide is repeatedly administered in increasing doses to induce the preferential expansion of antigen-specific T_{reg} s. Attempts to improve the efficacy of self-antigen tolerance include coupling antigen to autologous cells before injection. Polyclonal T_{reg} s can be expanded in vivo by signaling through the IL-2 receptor (CD25), which is constitutively expressed on T_{reg} s. Attempts to improve the efficacy of this approach in animal models have used recombinant IL-2 and monoclonal anti-IL-2 antibody immune complexes. The overall goal of both ex vivo and in vitro

approaches is to increase the relative T_{reg} -to- T_{eff} ratio in an attempt to restore tissue-specific or systemic immune homeostasis.

Table 1

Selected clinical trials using costimulatory blockade, T_{regs}, antigen-SIT, or IL-2 to treat human autoimmunity. *n*, number of patients enrolled; NCT number, ClinicalTrials.gov identifier; European trials, EudraCT number on.

Disease	Biologic	Phase	<i>n</i>	Objective/outcome	Reference
Costimulatory blockade					
Early erosive rheumatoid arthritis	CTLA 4-Ig (abatacept)	3b	459	Abatacept + methotrexate resulted in greater sustainable clinical, functional, and radiographic benefits (55.2% in remission at 2 years) than methotrexate alone, with acceptable safety and tolerability	(60)
Psoriatic arthritis	CTLA 4-Ig (abatacept)	2	170	American College of Rheumatology 20% criteria for improvement (ACR20) significantly higher than placebo (48% versus 19%) at a dose of 10 mg/kg	(7)
Type 1 diabetes	CTLA 4-Ig (abatacept)	2	112	Pancreatic insulin production was 59% higher at 2 years with abatacept (<i>n</i> = 73, 0.378 nM) compared to placebo (<i>n</i> = 30, 0.238 nM). No increased infections or neutropenia.	(8)
Multiple sclerosis	CTLA 4-Ig (abatacept)	1	20	Single CTLA-4-Ig infusion (16 patients) or four doses (4 patients). Reduction in myelin basic protein (MBP)-specific T cell proliferation within 2 months of infusion and decreased interferon- γ production by MBP-specific lines. Mild side effects.	(9)
Alopecia totalis/universalis	CTLA 4-Ig (abatacept)	1	~64	Test the safety and efficacy of abatacept in the treatment of alopecia totalis or alopecia universalis (not yet started)	NCT01314495
Multiple sclerosis	CTLA 4-Ig (abatacept)	2	~123	Evaluate the safety and efficacy of abatacept in adults with relapsing-remitting multiple sclerosis (recruiting)	NCT01116427
Systemic lupus erythematosus	CTLA 4-Ig (abatacept)	2b	118	Primary end point: proportion of patients with new flare was not met compared to placebo; increased serious adverse effects in abatacept group (19.8% versus 6.8%)	(10)
Systemic lupus erythematosus	Fully human mAb that binds to B7-related protein, B7RP-1 (AMG 557)	1	~48	Placebo-controlled, ascending, multiple-dose study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of AMG 557 in adults with systemic lupus erythematosus (recruiting)	NCT00774943
Phase 1 test in six healthy individuals	Agonistic anti-CD28 mAb (TGN1412; TeGenero)	1	6	Serious adverse affects resulting in multiorgan failure	(61)
Plaque psoriasis	Humanized mAb that binds to the α subunit (CD11a) of LFA-1 (efalizumab)	3	597	Efalizumab therapy resulted in significant improvements in plaque psoriasis in subjects with moderate-to-severe disease; drug withdrawn from the market due to an increased incidence of progressive multifocal leukoencephalopathy	(62, 63)

Disease	Biologic	Phase	n	Objective/outcome	Reference
Proliferative lupus glomerulonephritis	Humanized anti-CD40L antibody (BG9588)	2	28	A short course of BG9588 treatment reduced anti-dsDNA (double-stranded DNA) antibodies, increases C3 concentrations, and decreases hematuria; study was terminated prematurely because of thromboembolic events.	(64)
Regulatory T cell therapy					
GVHD in HSCT (in lymphoma patients)	Purified CD4 ⁺ CD25 ⁺ from haplotype-mismatched donor	Case series	28	T _{regs} prevented GVHD in the absence of posttransplant immunosuppression	(31)
Acute/chronic GVHD	Ex vivo-expanded CD4 ⁺ CD127 ⁺ CD25 ⁺ polyclonal T _{regs} from HLA-matched family donors	Case study	2	Chronic GVHD: significant alleviation of the symptoms and reduction of pharmacologic immunosuppression Grade IV aGVHD: therapy only transiently improved disease	(65)
aGVHD	Ex vivo-expanded partially HLA-matched third-party UCB CD25 ⁺ polyclonal T _{regs}	1	23	Objective: studying the side effects and best dose of donor T _{regs} after an UCB transplant in treating patients with advanced hematologic cancer or other disorder. Compared with identically treated historical controls without T _{regs} , modest reduction in incidence of grade II to IV aGVHD with no deleterious effect on risks of infection, relapse, or early mortality.	(33)
aGVHD	Freshly isolated donor T _{regs}	1	9	Safety and feasibility of T _{reg} transfer in patients with high risk of leukemia relapse after SCT. No adverse effects of infusion, no opportunistic infections or early disease relapses.	(66)
Type 1 diabetes	Ex vivo-expanded autologous CD4 ⁺ CD127 ^{lo/-} CD25 ⁺ polyclonal T _{regs}	1	~14	Assess the safety and feasibility of intravenous infusion of ex vivo-selected and ex vivo-expanded autologous polyclonal T _{regs} . Will also assess the effect of T _{regs} on β cell function as well as on other measures of diabetes severity and the autoimmune response underlying type 1 diabetes mellitus (recruiting)	NCT01210664
Antigen-SIT					
Multiple sclerosis (relapsing-remitting subtype)	Autologous peripheral blood mononuclear cells (PBMCs) coupled with seven immunodominant myelin peptides	1/2	9 (estimated)	Assess the safety, tolerability, and preliminary efficacy and in vivo mechanisms of action of intravenous administration of autologous PBMCs chemically coupled with myelin peptides (ongoing)	NCT01414634
Systemic lupus erythematosus	Spliceosomal peptide P140 (IPP-201101)	2	20	Three doses of 200 μ g of peptide given subcutaneously significantly improved status (reduced IgG anti-dsDNA antibody, reduced disease activity scores and SLE disease activity index)	(41)
Type 1 diabetes mellitus	Oral human insulin	3	300	Ongoing, prevention of type 1 diabetes in at-risk patients under 18	Europe (IT, FI, GB) 2006-006550-96
Type 1 diabetes mellitus (early-onset patients, <100 days diagnosed)	rhGAD65 formulated in alum	2	145	Non-efficacious in preventing disease progression	(67)

Disease	Biologic	Phase	n	Objective/outcome	Reference
Type 1 diabetes mellitus	rhGAD65 formulated in alum (DIAPREV-IT)	2	50	Prevention of type 1 diabetes in at-risk children (ongoing)	Sweden 2008-007484-16
Type 1 diabetes mellitus	Recombinant human insulin oral/intranasal	2	40	Prevention of type 1 diabetes in at-risk children under 18 (ongoing)	Europe (AT, GB) 2005-001621-29
Type 1 diabetes mellitus	A major T cell epitope of heat shock protein 60 (hsp60) (DiaPep277)	2	146	Preserves insulin production in adults with low- and moderate-risk HLA genotypes	(44)
IL-2 pathway					
Chronic GVHD	Low-dose subcutaneous IL-2	1	29	Favorable safety profile with suppression of disease	(54)
Steroid-refractory aGVHD	Combinations of low-dose IL-2, low-dose Vidaza, cyclophosphamide, and sirolimus	1/2	~15	Test the ability of low-dose IL-2 in addition to Vidaza, cyclophosphamide, and sirolimus in promoting and stabilizing the Foxp3 expression of T _{regs} (recruiting)	NCT01453140
GVHD	Low-dose IL-2	2	~36	Prophylaxis of GVHD after allogeneic HSCT (recruiting)	NCT00539695
Type 1 diabetes	Low-dose IL-2 (aldesleukin)	1/2	~24	Induce/stimulate T _{regs} in type 1 diabetes patients (IL-2 versus placebo) (recruiting)	NCT01353833
Type 1 diabetes	IL-2 and sirolimus (rapamycin)	1	10	Recent-onset type 1 diabetes (diagnosed within the previous 3 to 48 months); trial stopped (publication pending)	NCT00525889