Manipulating regulatory T cells: a promising strategy to treat autoimmunity

CD4⁺CD25⁺Foxp3⁺regulatory T cells (Treg cells) are extremely important in maintaining immune tolerance. Manipulation of Treg cells, especially autoantigen-specific Treg cells is a promising approach for treatments of autoimmune disease since Treg cells may provide the advantage of antigen specificity without overall immune suppression. However, the clinical application of Treg cells has long been limited due to low numbers of Treg cells and the difficulty in identifying their antigen specificity. In this review, we summarize studies that demonstrate regression of autoimmune diseases using Treg cells as therapeutics. We also discuss approaches to generate polyclonal and autoantigen-specific Treg cells *in vitro* and *in vivo*. We also discuss our recent study that describes a novel approach of generating autoantigen-specific Treg cells *in vivo* and restoring immune tolerance by two steps apoptosis–antigen therapy.

Keywords: antigen-specific Treg • autoantigen • autoimmune disease • cell apoptosis • IL-2 • immunotherapy • TGF- β • Treg

The immune system establishes a series of defense mechanisms to resist infection from a wide range of microorganisms. In healthy individuals, the immune system eliminates foreign antigens efficiently without attacking their own tissues, as the self-reactive cells that pose an immediate threat to autoimmunity were eliminated or inactivated by multiple mechanisms of immunological tolerance [1]. However, when there is a dysregulated control of self-reactive T cells, the immune response may also be launched against its own tissues, leading to the development of autoimmune disease.

Treg cells that express the alpha chain of the IL-2 receptor (CD25) and transcription factor forkhead box P3 (Foxp3) are a subpopulation of CD4⁺ T cells that maintain tolerance to self-antigens by regulating other types of immune cells. It is well known that Treg cells are generated in the thymus (thymus-derived Treg cells, tTreg cells) and can also be induced from naive T cells in the periphery (peripheral-induced Treg cells, pTreg cells). Treg cells have been considered the major

mediator of peripheral tolerance ever since it was reported that transfer of CD25-depleted peripheral CD4⁺ T cells produced a variety of autoimmune diseases in nude mice, whereas cotransfer of CD4⁺CD25⁺ T cells suppressed the development of autoimmunity [2]. The potent immunosuppressive effects of Treg cells have led to a possibility of harnessing this cell population to treat autoimmunity. In this review, we focus on studies that demonstrate treatment of autoimmune diseases with Treg cells. We also compare the different approaches to generate autoantigen-specific Treg cells.

Polyclonal Treg cells versus autoantigen-specific Treg cells in the treatment of autoimmunity

Several studies have shown that adoptive transfer of polyclonal Treg cells is able to prevent the development of autoimmune diseases [3–10]. However, the therapeutic effects of polyclonal Treg cells on established diseases are much poorer. Since patients with autoimmune disease have dysregulated immune

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responses, the major objective for clinical application of Treg cells is to reverse or cure established disease [11]. Administration of polyclonal Treg cells was shown to be effective in reversing established collagen-induced arthritis in lethally-irradiated syngeneic BM grafted DBA/1 mice and colitis in RAG^{-/-} mice, but had little effect in immunocompetent mice unless much higher numbers of Treg cells were used [12,13]. The most promising approach to generate polyclonal Treg cells for treatment so far, is administration of low doses of IL-2 (in soluble form or IL-2 coupled to anti-IL-2 monoclonal antibody), which expands the Treg population and enhances tolerance *in vivo* [14–16]. Nevertheless, the use of polyclonal Treg cells expanded *ex vivo* or *in vivo* poses the potential risk of inducing overall immune suppression and compromising beneficial immune responses.

By contrast, autoantigen-specific Treg cells may provide the advantage of antigen specificity without overall immune suppression, and thus are a better alternative for the treatment of autoimmune disease [17–21]. A number of studies have shown that autoantigen-specific Treg cells are able to prevent the development of autoimmune diseases [22–33]. Moreover, there are cases where established autoimmune diseases are reversed when diseased mice are treated with autoantigen-specific Treg cells (Table 1) [23,34–39]. Studies in the NOD mouse model demonstrate that islet antigen-specific

Treg cells were far more potent than polyclonal Treg cells in preventing the onset of diabetes. Importantly, only the transfer of autoantigen-specific Treg cells but not polyclonal Treg cells, was able to suppress ongoing diabetes [23,24,35,39]. Therefore, compared with polyclonal Treg cells, autoantigen-specific Treg cells represent an attractive and promising therapeutic approach for treatment of autoimmune diseases.

Approaches to generate Treg cells *in vitro* and *in vivo*

In the past many years, the low frequency of Treg cells in the periphery was one of the major challenges for developing Treg cell-based immunotherapy. However, recent advancement in the understanding of Treg cell generation and expansion, has led to the development of approaches to generate Treg cells *in vitro* and *in vivo*. Treg cells generated by these approaches have achieved exciting result in preclinical research and may potentially have clinical application.

Expansion of polyclonal Treg cells *in vivo*

During the past years, many approaches have been discovered to expand polyclonal Treg cells to treat autoimmune diseases, and great advances have been made in clinical trials. Although these treatments expand polyclonal Treg cells but not autoantigen-specific Treg cells, which have the risk of inducing overall immune

Table 1. Summary of autoimmune disease treatment with antigen-specific Treg cells.			
Study (year)	Disease	Approach to generate Treg cells	Ref.
Jaeckel et al. (2005)	Type 1 diabetes	TCR transgenic antigen-specific T cells transduced with <i>FoxP3</i> gene <i>in vitro</i>	[39]
Tang et al. (2004)	Type 1 diabetes	TCR transgenic antigen-specific Treg cells amplified by anti-CD3/ anti-CD28 plus IL-2 <i>in vitro</i>	[23]
Tarbell et al. (2007)	Type 1 diabetes	TCR transgenic antigen-specific Treg cells amplified by antigen plus dendritic cells <i>in vitro</i>	[35]
Kasagi et al. (2014)	Type 1 diabetes	Antigen-specific Treg cells induced in the disease onset individuals by apoptosis–antigen therapy <i>in vivo</i>	[34]
Nguyen et al. (2011)	Autoimmune gastritis	TCR transgenic antigen-specific Treg cells induced by anti-CD3/ anti-CD28 plus TGF-β <i>in vitro</i>	[36]
Tu et al. (2013)	Autoimmune gastritis	TCR transgenic antigen-specific Treg cells induced by peptide, irradiated APC and TGF-β <i>in vitro</i>	[38]
Kasagi et al. (2014)	Multiple sclerosis/EAE	Antigen-specific Treg cells induced in the disease onset individuals by apoptosis–antigen therapy <i>in vivo</i>	[34]
Stephens et al. (2009)	Multiple sclerosis/EAE	TCR transgenic antigen-specific Treg cells amplified by anti-CD3/ anti-CD28 plus IL-2 <i>in vitro</i>	[37]

suppression, they may represent a useful strategy to treat autoimmunity, especially when the antigens causing the disease are unknown or if the disease is caused by self-reactive T cells that recognize multiple antigens or epitopes.

Expansion by IL-2 and IL-2–anti-IL-2 complexes

It has been shown that administration of IL-2–anti-IL-2 complexes results in a selective expansion of Treg cells in several organs and confers resistance to EAE and acceptance of islet grafts [16]. Similarly, low-dose IL-2 therapy also increases the number of Treg cells *in vivo* and reverses recent-onset diabetes [15]. Moreover, low-dose IL-2 therapy has achieved promising results in treating Type 1 diabetes and other autoimmune diseases in clinical trials [14,40–42]. Apart from autoimmune diseases, It has been reported Treg cells generated by low-dose IL-2 or combination of IL-2 with rapamycin suppress graft versus host disease (GvHD) [43,44]. In light of these studies, IL-2 and IL-2–anti-IL-2 complexes treatment represent a promising approach to expand Treg cells and treat autoimmunity (Figure 1). However, the dosage of IL-2 administered selection is crucial for the efficacy of treatment, as high-dose IL-2 treatment not only increases the number of Treg cells, but also enhance functions of pathogenic Teff cells, which may accelerate tissue destruction [45]. In addition to the expansion of Treg cells, IL-2 could also suppress autoimmunity through other mechanism. It was recently found that IL-2 prevented the developing of T-follicular helper (Tfh) cells, which expand in autoimmune disease patients and promote long-term effector B-cell responses [46].

Expansion by intravenous immunoglobulin

It has been demonstrated that Treg cells can be expanded by intravenous immunoglobulin (IVIg) [47,48]. IVIg is a therapeutic preparation of human IgG antibodies administered intravenously. Now IVIg has been widely used for the treatment of autoimmune diseases, such as immune thrombocytopenia, inflammatory diseases, Kawasaki disease and Guillain–Barre Syndrome. Recently, it has been demonstrated that IVIg expands Treg cells via induction of cyclooxygenase (COX)-2-dependent prostaglandin E2 (PGE2) in human dendritic cells (DCs) [47]. This uncovered cellular and molecular mechanism provides us a novel approach to expand Treg cells and regulate immune tolerance.

Expansion &/or induction by neutralizing inflammatory cytokines

It has been demonstrated that Treg cells may lose their suppressive activity or even convert to effector cells when immune cells are uncontrollably acti-

vated with increased production of pro-inflammatory cytokines [34,49,50]. As a result, it may be possible to enhance Treg cell function through neutralization of pro-inflammatory cytokines *in vivo*. In fact, neutralization of inflammatory cytokines not only rescues Treg cell function, but also expands and/or induces Treg cells [51–54].

Expansion by rapamycin

The immunosuppressive drug rapamycin (RAPA), which has been used clinically to prevent graft rejection, suppresses T cell activation and proliferation [55]. Recently, a number of studies have demonstrated that RAPA promotes tolerance by promoting the expansion and survival of Treg cells and promoting the generation of induced Treg cells [55–58]. The ability of rapamycin to expand Treg cells and deplete T effector cells can be exploited for use in clinical settings, although global immune suppression may be a potential side effect.

Generation of autoantigen-specific Treg cells *in vitro*

Treatment with autoantigen-specific Treg cells provides the advantage of antigen specificity, and at the same time eliminates the risk of inducing overall immune suppression. Furthermore, *in vitro* produced Treg cells can be analysed phenotypically and functionally prior to infusion and Treg cell dosage can be precisely controlled. However, difficulties in identifying antigen specificity of Treg cells and in expansion of antigen-specific Treg cells to sufficient numbers for treatment have limited their clinical application in the past. More recently, a number of studies have reported the generation and expansion of autoantigen-specific Treg cells under *in vitro* conditions, making treatment of autoimmunity with autoantigen-specific Treg cells feasible (Figure 2).

Ex vivo expansion of autoantigen-specific Treg cells

It was demonstrated that islet-specific Treg cells purified from the BDC2.5 T-cell receptor (TCR) transgenic mice (BDC2.5 Treg cells) that were expanded *in vitro* by antigen-pulsed DCs were more suppressive than freshly isolated ones [24]. Furthermore, DCs-expanded BDC2.5 Treg cells potently suppressed the development of diabetes and even reversed established disease [24,35]. Several groups showed that stimulation with anti-CD3/anti-CD28-coated beads in the presence of high concentrations of IL-2 can also drive the expansion of autoantigen-specific Treg cells that were highly suppressive *in vivo* [23,37]. Nevertheless, these studies were based on the manipulation of TCR transgenic Treg cells, which is not applicable in the clinical setting.

Figure 1. A model of treatment with low-dose IL-2 or IL-2-anti-IL-2 complexes *in vivo*. Treatment with low-dose IL-2 or IL-2-anti-IL-2 complexes results in a selective expansion of Treg cells, but not effector T cells. So the autoantigen specific Treg cells were expanded together with all other polyclonal Treg cells. The expanded autoantigen-specific Treg cells can suppress the autoantigen effector T cells better and reverse the disease.

Generation of autoantigen-specific Treg cells by retroviral infection

Studies have shown that autoantigen-specific Treg cells can also be generated by infecting naive T cells with retrovirus that carries both Foxp3 and TCR transgenes or infecting polyclonal Treg cells with retrovirus that carries the TCR transgene only. Both populations of genetically-modified Treg cells have been shown to be effective in suppressing the development of arthritis [33]. This may represent a potential approach to generate autoantigen-specific Treg cells for clinical application. However, these cells retain characteristics of polyclonal Treg cells, which may compromise otherwise beneficial immune responses. Moreover, the biosafety of integrative viral vectors remains to be fully assessed.

Induction of autoantigen-specific Treg cells by TGF- β *in vitro*

Stimulation of naive CD4⁺ T cells in the presence of TGF- β is able to convert them into Treg cells (induced regulatory T cells, iTreg cells), which exhibits suppressive ability *in vitro* and *in vivo* [29,31,36,59], and is a widely used approach in preclinical research. The major challenge of using iTreg cells as treatment comes from their unstable Foxp3 expression and suppressive activity. This occurs upon antigen stimulation in the absence of TGF- β as the result of heavy methylation of the CpG motifs within the Foxp3 locus [60–62]. Although retinoic acid has been shown to stabilize Foxp3 expression, even in the presence of pro-inflammatory cytokines [49,63], the Foxp3 enhancer region remains methylated. This finding suggests that iTreg cells are not as stable as thymus-derived Treg (tTreg) cells, as

complete demethylation is required for stable Foxp3 expression [64–66]. Nevertheless, similar to expanding autoantigen-specific Treg cells *in vitro*, these studies generated autoantigen-specific Treg cells using TCR transgenic T cells. Therefore, the application of this approach may also be limited in the clinical setting.

Generation of autoantigen-specific Treg cells *in vivo*

The discovery that Treg cells can be induced from naive T cells in the periphery (pTreg cells) by TGF- β gives rise to a possibility that autoantigen-specific Treg cells can be induced *in vivo* [59,67,68].

Induction of autoantigen-specific Treg cells by antigen plus DCs *in vivo*

It is well demonstrated that autoantigen-specific Treg cell can be induced *in vivo* by low-dose antigen delivery, which is TGF- β dependent [69,70]. Additionally, injection of DCs pulsed with antigens or targeting of antigens to immature DCs by DEC-205 antibody have been shown to induce the generation of autoantigen-specific Treg cells *in vivo* and prevent the development of autoimmunity [70–74]. However, its therapeutic efficacy on established diseases has not been established. Since autoantigen-responsive immune cells in diseased state are uncontrollably activated with increased production of pro-inflammatory cytokines, autoantigen-specific Treg cells may lose their suppressive activity or even convert to effector cells in such an environment [34,49]. Treg cells may even be converted into Th17 cells in inflammatory conditions [49,75–78]. Moreover, as we have discussed in an earlier part of the review,

Treg cells can be expanded by neutralizing inflammatory cytokines. Therefore, combining of antigen plus DCs treatment with neutralization of inflammatory cytokines may achieve better therapeutic results.

Induction of autoantigen-specific Treg cells by antigen pulsed ethylene carbodiimide fixed cell therapy *in vivo*

During the past years, treatment of autoimmunity and transplantation immunity with antigen pulsed ethyl-

ene carbodiimide (ECDI)-fixed cells has been extensively tested in animal models and a Phase I trial of multiple sclerosis [79–83]. The antigen pulsed ECDI-fixed cells therapy is an intravenous infusion of autoantigens cross-linked to the surface of splenic antigen-presenting cells (Ag-APC), peripheral blood (Ag-PBL) or splenic leukocytes (Ag-SP) using ECDI, which affects antigen coupling and induce cellular apoptosis [81,83]. The possible mechanism may be dependent on TGF- β production by both phagocytes and apop-

Figure 2. Approaches to generate autoantigen-specific Treg cells *in vitro*. Autoantigen-specific Treg cells can be expanded by antigen-pulsed dendritic cells or anti-CD3/anti-CD28-coated beads in the presence of high concentrations of IL-2 (A), or generated by infecting naive T cells with retrovirus that carries both Foxp3 and TCR transgenes (B) or infecting polyclonal Treg cells with retrovirus that carries the TCR transgene only (C), or induced by culturing naive T cells with TCR stimulation (antigen plus antigen-presenting cell or anti-CD3/anti-CD28), TGF- β and RA (D).

RA: Retinoic acid; Teff: T effector.

Figure 3. A model of apoptosis-antigen therapy *in vivo*. Apoptosis of immune cells was first induced by antibody depletion or γ -irradiation. Apoptotic cells are then detected and digested by phagocytes, during which TGF- β is produced by both phagocytes and apoptotic cells. Following administration of self-peptides, naive T cells initiate TCR signaling within this TGF- β -rich environment, and Foxp3 expression is induced to instruct naive T cells along the Treg differentiation pathway. These induced Treg cells act to maintain tolerance toward autoantigen and reverse autoimmune disease.

otic cells [84,85], as ECDI induces cell apoptosis and apoptotic cells are indispensable in this treatment [83]. Indeed, it has been demonstrated that TGF- β plays an important role in the Ag-coupled apoptotic cell therapy through induction of iTreg cells [82–83,86]. It is an efficient approach to induce autoantigen-specific Treg cells *in vivo*, which has achieved exciting preclinical results in mouse models of multiple sclerosis, Type 1 diabetes and transplantation [83].

On the other hand, it has been shown that autoantigen-specific Treg cell treatment resulted in the functional repair and restitution of tissue that had been severely damaged by inflammation, however, auto-reactive effector/memory T cells persisted in treated mice, resulting in residual cellular infiltrates within the repaired stomach tissue, presenting the risk of relapse [38]. So a better approach to target established

autoimmune disease may rely on correcting the dys-regulated immune system by reducing the infiltrating pathogenic T cells before generation of antigen-specific Treg cells, in doing so, reprogramming immune tolerance.

Induction of autoantigen-specific Treg cells by apoptosis-antigen therapy *in vivo*

Our group have recently developed a novel approach to induce autoantigen-specific Treg cells *in vivo* that demonstrate therapeutic effects in mouse models of autoimmunity [34]. We found that the immune tolerance could be restored in mice with autoimmunity with a combination of immune cell apoptosis followed by administration of self-peptides to induce the generation of autoantigen-specific Treg cells. The apoptosis of immune cells was induced by γ -irradiation or

Executive summary

Background

- In healthy individuals, the immune system eliminates foreign antigens efficiently without attacking their own tissues.
- When there is a dysregulated control of self-reactive T cells, the immune response may also be launched against its own tissues, leading to the development of autoimmune disease.
- Treg cells have been considered the major mediator of peripheral tolerance. The potent immunosuppressive effects of Treg cells have led to a possibility of harnessing this cell population to treat autoimmunity.

Polyclonal Treg cells versus autoantigen-specific Treg cells in the treatment of autoimmunity

- The therapeutic effects of polyclonal Treg cells on established diseases are much poorer than prevention of autoimmune diseases, and the use of polyclonal Treg cells poses the potential risk of inducing overall immune suppression and compromising beneficial immune responses.
- Autoantigen-specific Treg cells are able to reversed established autoimmune diseases, and may provide the advantage of antigen specificity without overall immune suppression.

Expansion of polyclonal Treg cells *in vivo*

- IL-2–anti-IL-2 complexes, low-dose IL-2 and combination of IL-2 with rapamycin promote expansion of Treg cells. These approaches suppress autoimmunity and transplants immunity in both animal models and clinical trials.
- Intravenous immunoglobulin, a therapeutic preparation of human IgG antibodies administered intravenously, was found to expand Treg cells via induction of cyclooxygenase-2 dependent prostaglandin E2 in human dendritic cells (DCs).
- Neutralization of inflammatory cytokines not only rescues Treg cell functions, but also expand and/or induce Treg cells.
- Rapamycin not only suppresses T cell activation and proliferation, but also promote tolerance by promoting the expansion and survival of Treg cells and generation of induced Treg cells.

Generation of autoantigen-specific Treg cells *in vitro*

- TCR transgenic Treg cells can be expanded *in vitro* by antigen-pulsed DCs or anti-CD3/anti-CD28-coated beads plus high concentrations of IL-2. Stimulation of TCR transgenic naive CD4⁺ T cells in the presence of TGF- β is able to convert them into Treg cells. Nevertheless, these studies were based on the manipulation of TCR transgenic Treg cells, which is not applicable in the clinical setting.
- Autoantigen-specific Treg cells can also be generated by infecting naive T cells with retrovirus that carries both Foxp3 and TCR transgenes or infecting polyclonal Treg cells with retrovirus that carries the TCR transgene only. This may represent a potential approach to generate autoantigen-specific Treg cells for clinical application. However, the biosafety of integrative viral vectors and the risk of beneficial immune responses suppression limit the clinical application.

Generation of autoantigen-specific Treg cells *in vivo*

- Autoantigen-specific Treg cell can be induced *in vivo* by low-dose antigen delivery or injection of DCs pulsed antigens, which is TGF- β dependent, and prevents the development of autoimmunity.
- Antigen pulsed ethylene carbodiimide (ECDI)-fixed cell treatment, which is ECDI-induced cell apoptosis and TGF- β dependent, is an efficient approach to induce autoantigen-specific Treg cells *in vivo*. It has got extensively developed in animal models and a Phase I trial. However, autoreactive effector/memory T cells persisted in autoantigen-specific Treg cell treated mice, resulting in residual cellular infiltrates within the repaired stomach tissue, presenting the risk of relapse.
- A combination of immune cell apoptosis followed by administration of self-peptides was a better approach to induce the generation of autoantigen-specific Treg cells and restore the immune tolerance. This novel apoptosis–antigen therapy reduces levels of inflammatory cytokines released by immune cells, which then ensures the further induction of Treg cells in a TGF- β rich immunoregulatory milieu. This therapy not only generates antigen-specific Treg cells, but also reduces the number of infiltrating effector T cells, thus lowering the risk of relapse.

Conclusion & future perspective

- Among the approaches to generate autoantigen-specific Treg cells, low-dose IL-2 therapy, antigen pulsed ECDI-fixed cell therapy and the two-step apoptosis–antigen therapy have achieved exciting results. The apoptosis–antigen therapy showed long-term immune tolerance to autoantigens without dampening beneficial immune responses, which will benefit patients with autoimmune diseases.
- Treg cell therapy, especially with autoantigen-specific Treg cells, will become a reliable approach to treat autoimmunity, but how to translate Treg treatment to clinical application need deep research.

Executive summary (cont.)

Conclusion & future perspective (cont.)

- Future work should investigate identification of autoantigens for the generation of antigen-specific Treg cells, generation of autoantigen specific Treg cells in more suave manners (e.g., oral tolerance) and dose choice of Treg cells and antigens to treat autoimmunity amongst individuals and different diseases. Surmounting these challenges is crucial for designing safe and effective treatments in various autoimmune diseases.

antibody depletion. Apoptotic cells were detected and digested by phagocytes, after which TGF- β is produced by both phagocytes and apoptotic cells in this process [84,85]. This novel apoptosis–antigen therapy reduces levels of inflammatory cytokines released by immune cells, which then ensures the further induction of Treg cells in a TGF- β rich immunoregulatory milieu (Figure 3) [34].

This therapy not only generates antigen-specific Treg cells, but also reduces the number of infiltrating effector T cells, thus lowering the risk of relapse. Importantly, this approach can also be applied in a clinical setting, as both anti-CD20 antibody depletion and γ -irradiation have been used in patients with autoimmunity for clinical treatment. Together with irradiation and antibody treatment, the administration of self-peptides could be the compound two-step apoptosis–antigen therapy for patients and may induce long-term tolerance.

Conclusion & future perspective

Treg cells were found to be critical in maintaining self-tolerance 20 years ago [2]. Since then, significant progress has been made in manipulating polyclonal and antigen-specific Treg cells to treat autoimmunity. The findings in both preclinical researches and clinical trials using polyclonal Treg cells or autoantigen-specific Treg cell therapy have shown great potential in clinical application [11,14,20]. Importantly, unlike immunosuppressive drugs that induce global immunosuppressive effects, autoantigen-specific Treg cells may provide the advantage of antigen specificity without overall immune suppression. However, further studies are needed to investigate how to translate Treg treatment, especially with autoantigen-specific Treg cells, into clinical settings. Among the approaches to generate autoantigen-specific Treg cells, low-dose IL-2 therapy, antigen pulsed ECDI-fixed cell therapy and the two-step apoptosis–antigen therapy have achieved exciting results. The apoptosis–

antigen therapy showed long-term immune tolerance to autoantigens without dampening beneficial immune responses, which will benefit patients with autoimmune diseases [34]. Overall, based on what have been discovered and what we can predict, Treg cell therapy, especially with autoantigen-specific Treg cells, will become a reliable approach to treat autoimmunity.

Although great advances have been made in both preclinical researches and clinical trials, there are still many obstacles to overcome, such as identification of autoantigens for the generation of antigen-specific Treg cells, generation of autoantigen specific Treg cells in more suave manners (e.g., oral tolerance) and how to choose the optimal doses of Treg cells and antigens to treat autoimmunity among individuals and different diseases. Surmounting these challenges is crucial for designing safe and effective treatments for various autoimmune diseases.

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