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REVIEW

Role of TGF-β in Self-Peptide Regulation of Autoimmunity

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Abstract Transforming growth factor (TGF)-β has been implicated in regulation of the immune system, including autoimmunity. We have found that TGF-β is readily produced by T cells following immunization with self-peptide epitopes that downregulate autoimmune responses in type 1 diabetes (T1D) prone nonobese diabetic (NOD) mice. These include multiple peptide epitopes derived from the islet β-cell antigens GAD65 (GAD65 p202-221, GAD65 p217-236), GAD67 (GAD67 p210-229, GAD67 p225-244), IGRP (IGRP p123-145, IGRP p195-214) and insulin B-chain (Ins. B:9-23) that protected NOD mice from T1D. Immunization of NOD mice with the self-MHC class II I-A^{g7} β-chain-derived peptide, I-$A\beta^{g7}$ p54-76 also induced large amounts of TGF-β and also protected these mice from diabetes development. These results indicate that peptides derived from disease related self-antigens and MHC class II molecules primarily induce TGF-β producing regulatory Th3 and Tr1-like cells. TGF-β produced by these cells could enhance the differentiation of induced regulatory iTreg and iTreg17 cells to prevent induction and progression of autoimmune diseases. We therefore suggest that peripheral immune tolerance could be induced and maintained by immunization with self-peptides that induce TGF-β producing T cells.

Keywords Auto-antigens · NOD mice · Peptide epitopes · Regulatory T cells · TGF-beta · Type 1 diabetes

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Introduction

Transforming growth factor (TGF)-β plays an important role in many pathological and physiological responses and has been implicated in the regulation of adaptive and innate immunity (Letterio and Roberts 1998; Li et al. 2006; You et al. 2006). Induction of regulatory and effector T cells such as Treg and Th17 cells, secretion of antibodies by B cells and inhibition of innate immune cells such as NK cells could be modulated by TGF-β (Travis and Sheppard 2014). In addition, TGF-β is involved in the proliferation and differentiation of thymic T cells and in the homeostasis of peripheral T cells (Stritesky et al. 2012).

In mammals, TGF-β is present in three isoforms: TGF-β1, TGF-β2 and TGF-β3. These molecules are structurally very similar and are secreted by immune and non-hematopoietic cells. TGF-β acts through specific heterodimeric TGFβRI and TGFβRII receptors on cells through Smad-dependent and Smad-independent pathways (Derynck and Zhang 2003). Since T cells are the major targets of TGF-β, it plays a crucial role in immune tolerance and in maintaining immune homeostasis by inhibiting the proliferation, differentiation, activation and effector function of pathogenic T cells. Because of these immunoregulatory properties, TGF-β has significant potential in the treatment of autoimmune diseases (Sanjabi et al. 2017).

In autoimmune diseases such as type 1 diabetes (T1D), TGF-β inhibits the proliferation of effector T cells as well as inflammatory cytokine production from these cells. TGF-β has both pro- and anti-inflammatory properties and plays an essential role in establishing immunological tolerance. On its own, TGF-β induces $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells while in the presence of interleukin (IL)-6 it induces IL-17-producing regulatory Treg17 cells. However, in the presence of IL-23 and IL-6 it induces highly pathogenic Th17 cells (Bellemore et al. 2015; Singh et al. 2013).

Initially Th2 cells were considered to be inhibitory cells in controlling autoimmune diseases mediated by Th1 cells (Bach 1994; Delovitch and Singh 1997). However, with the discovery of Treg cells, the concept of the regulation of autoimmunity has completely changed (Bluestone et al. 2008; Gomez-Tourino et al. 2016; Sakaguchi 2004; Walker and von Herrath 2016). Another class of Treg cells, namely Tr1 and Th3 cells has also been shown to effectively block autoimmune diseases such as T1D (Zeng et al. 2015). These cells produce large amounts of TGF-β and were shown to be involved in preventing T1D in nonobese diabetic (NOD) mice (Battaglia et al. 2006a, b; Han et al. 1996, 1997). Recently Tr1 cells were shown to be induced by antigen/MHC carrying nanoparticles (Clemente-Casares et al. 2016; Serra and Santamaria 2015). These nanoparticles effectively downregulate or prevent several autoimmune diseases in experimental model systems (Clemente-Casares et al. 2016).

We have explored whether TGF-β-producing Treg cell subsets are induced by islet β-cell-associated antigenic peptides that have demonstrated the potential to delay or prevent T1D. We used several antigenic peptides of β-cell antigens, namely GAD65, GAD67, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) and insulin B-chain for these studies (Mukherjee et al. 2003b, 2005; Zechel et al. 1997, 1998a, b). These peptides prevent or delay T1D development in NOD mice by inducing TGF-β-producing T cells. We previously reported that a peptide of MHC class II β-chain, namely I-$A\beta^{g7}$ p54-76, prevented T1D in NOD mice and induced Tr1 cells that secrete a large amount of TGF-β (Mukherjee et al. 2003a). Although the mechanism is not well understood, TGF-β induction appears to play an essential role in preventing T1D in NOD mice (Han et al. 1996, 1997). The induction and maintenance of Treg cells have been considered to be essential features in peripheral immune tolerance. Our results suggest that TGF-β-producing Th3 and Tr1 cells are likely to be more important in disease prevention and regulating autoimmunity (Li et al. 2007; Pot et al. 2011).

GAD65 and GAD67 Peptides Induce TGF-β-Producing Th3 Cells and Inhibit Diabetes Development in NOD Mice

A number of different autoantigens have been identified in the development and progression of T1D (Bach and Chatenoud 2001; Delong et al. 2013; Di Lorenzo et al. 2007; Mallone et al. 2011; Roep and Peakman 2012; Zechel et al. 1997). Several studies have demonstrated that glutamic acid decarboxylase (GAD) is an important autoantigen involved in the modulation of T1D (Elliott et al. 1994; Jun et al. 2002; Lernmark and Larsson 2013; Yang et al. 2013a). Studies in the NOD mouse have shown that several immunodominant GAD epitopes participate in the disease-causing inflammatory response that leads to the eventual destruction of the β cells (Kaufman et al. 1993, 2001; Kim et al. 2004; Quinn et al. 2001; Tisch et al. 1993, 2001; Zechel et al. 1998a, b). High levels of pro-inflammatory cytokine production by antigen-specific Th1 cells characterize this response. Several previous studies, including our own, have reported that intraperitoneal (i.p.) immunization with a number of GAD peptides reduced diabetes in NOD mice (Kaufman et al. 1993; Kim et al. 2004; Zechel et al. 1997, 1998b). This was likely mediated by the induction of cytokines produced by Treg cells. Other studies have also demonstrated that a panel of autoreactive peptides in T1D, such insulin B-chain peptide B9-23 (Mukherjee et al. 2003b) or GAD65 p524-543, given to NOD mice enhanced the production of IL-10 and TGF-β resulting in suppression of autoimmune diabetes (Maron et al. 1999).

GAD Peptides GAD65 p202-221, GAD65 p217-236 and GAD67 p210-229, GAD67 p225-244 Inhibit Diabetes Incidence in NOD Mice

To determine the protective efficacy of a panel of GAD65 and GAD67 peptides that we previously identified (Zechel et al. 1998a) (Table 1), 4-week-old female NOD/Ltj mice were immunized i.p. with 100 μg of GAD peptide emulsified in incomplete Freund's adjuvant (IFA)/saline. As outlined in Table 2, these GAD peptides reduced the diabetic incidence in these mice by 30 weeks of age. The disease incidence varied with each peptide and ranged from 10% incidence for GAD67 p210-229, 30% for GAD67 p225-244 to 50% for GAD65 p202-221 and 40% for GAD65 p217-236. All the IFA/saline-treated control mice developed diabetes by 30 weeks of age. In addition, statistical determination of the protective efficacy of each peptide revealed that all peptides demonstrated significant levels of protection as compared to the IFA/saline control ($p < 0.05$).

Table 1 Sequences of autoantigenic peptides that induced TGF-β producing $CD4^+$ T cells

Peptides	Sequence	References
GAD65 peptides		
GAD65 p202-221	TNMFTYEIAPVFVLLEYVTL	Zechel et al. (1998a, b)
GAD65 p217-236	EYVTLKKMREIIGWPGGSGD	
GAD67 peptides		
GAD67 p210-229	TNMFTYEIAPVFVLMEQITL	Zechel et al. (1998a, b)
GAD67 p225-244	EQITLKKMREIVGWSNKDGD	
Insulin B-chain peptide		
Ins. B:9-23	SHLVEALYLVCGERG	Daniel and Wegmann (1996), Mukherjee et al. (2003b)
IGRP peptides		
IGRP p123-145	WYVMVTAALSYTISRMEESSVTL	Mukherjee et al. (2005)
IGRP p195-214	HTPGVHMASLSVYLKTNVFL	
I-Aβ^{g7} peptide		
I-Aβ^{g7}p54-76	GRHSAEYYNKQYLERTRAELDTA	Chaturvedi et al. (2000), Mukherjee et al. (2003a)

Table 2 $CD4^+$ T cell subset induced by autoantigenic peptides in NOD mice[a]

Autoantigenic peptide epitopes[a]	% Diabetes at 30 weeks of age[b]	IFN-γ[c] (pg/ml)	TGF-β[c] (pg/ml)	IL-4[c] (pg/ml)	IL-10[c] (pg/ml)	Th cell subset induced[d]
GAD65 p202-221	50	10,000 ± 2670	1050 ± 30	<1	200 ± 30	Th3
GAD65 p217-236	10	8700 ± 700	1100 ± 266	<1	190 ± 25	Th3
GAD67 p210-229	60	7100 ± 700	825 ± 151	<1	0	Th3
GAD67 p225-244	30	5100 ± 1772	805 ± 85	<1	0	Th3
Insulin B:9-23	20	78 ± 5	530 ± 30	<1	<5	Th3
IGRP p195-214	10	1604 ± 98	858 ± 55	8 ± 1	102 ± 10	Th3
IGRP p123-145	30	2551 ± 161	23 ± 9	99 ± 4	725 ± 25	Th2
I-Aβ^{g7} p54-76	30	400 ± 25	950 ± 90	45 ± 10	3970 ± 122	Tr1
I-Aβ^{g7} p54-76 cell line (I-A.Y)	0	<10	4150 ± 75	280 ± 50	17,500 ± 200	Tr1

[a] Methods: female NOD/Ltj mice (3- to 4-week-old) were immunized with various type 1 diabetes-related autoantigenic peptides (Table 1) in IFA/saline as previously described and were monitored for diabetes development until 30 weeks of age (Chaturvedi et al. 2000; Mukherjee et al. 2003a, b, 2005; Zechel et al. 1998a, b)

[b] The % diabetes incidence in autoantigenic peptide/IFA immunized NOD mice is indicated at 30 weeks of age. The disease incidence was significantly reduced in autoantigenic peptide/IFA immunized mice as compared to IFA/saline treated mice ($p < 0.05$). All IFA/saline-treated control mice developed diabetes by the age of 30 weeks. Adoptive transfer of I-Aβ^{g7}p54-76 cell line (I-A.Y) in NOD/SCID mice completely prevented disease transfer (Mukherjee et al. 2003a)

[c] Cytokines produced by antigen-specific $CD4^+$ T cells from the lymph nodes of various peptide-immunized (Table 1) NOD/Ltj mice or from I-Aβ^{g7}p54-76 specific I-A.Y cell line were measured by ELISA as previously reported (Chaturvedi et al. 2000; Mukherjee et al. 2003a, b, 2005; Zechel et al. 1998a, b)

[d] The designation of Th cell subsets induced by various autoantigenic peptides in NOD mice is based upon their cytokine profile e.g. Tr1: IL-10^{++} TGF-β^+; Th2: IL-4^+ IL-10^+ and Th3: TGF-β^{++}

Cytokine Analysis of GAD65 p202-221, GAD65 p217-236 and GAD67 p210-229, GAD67 p225-244 Peptide-Primed NOD T Cell Supernatants

A number of different cytokines have been shown to play an integral role in disease induction and modulation of autoimmune diabetes in NOD mice (Rabinovitch et al. 1997; Rabinovitch 2003; Rabinovitch and Suarez-Pinzon 2007; Suarez-Pinzon and Rabinovitch 2001). To determine the regulatory cytokine profile elicited by peptide-primed lymphocytes, we analyzed the culture supernatants of $CD4^+$ T cells from peptide immunized mice by ELISA.

Female NOD/Ltj mice aged 6–8 weeks were injected with 100 μg of GAD peptide emulsified in complete Freund's adjuvant. Ten days later, T cells were isolated from the draining lymph nodes, stimulated with GAD peptides for 72 h in vitro, and the culture supernatants analyzed for various cytokines by ELISA (Table 2). We found that GAD peptides induced secretion of high levels of interferon (IFN)-γ (5100–10,000 pg/ml) and almost no IL-4 (<1 pg/ml). To determine whether immunosuppressive cytokines were present in these culture supernatants, samples were also assayed for IL-10, TGF-β, and tumor necrosis factor (TNF)-α (Table 2). Both IL-10 and TGF-β have demonstrated the ability to down-regulate IFN-γ-mediated inflammatory responses (Rabinovitch 2003). In addition, TNF-α has been shown to protect NOD mice from the onset of T1D (Rabinovitch et al. 1997). As shown in Table 2, we found high levels of TGF-β but only low levels of IL-10 in these cultures. No TNF-α was detected in any of the assayed culture supernatants. The presence of low levels of IL-10 coupled with higher levels of TGF-β is characteristic of the Th3 cell profile. This suggests induction of Th3 regulatory T cells by the four GAD peptides used in our disease protection studies.

Insulin B-Chain, Ins. B:9-23 Peptide Induce TGF-β-Producing Th3 Cells and Inhibit Diabetes Development in NOD Mice

Previous studies have shown that insulin B chain, Ins. B:9-23 peptide is an important autoantigenic epitope involved in the modulation of T1D (Daniel and Wegmann 1996; Di Lorenzo et al. 2007; Zechel et al. 1997). Our work has identified the generation of antigen-specific regulatory $CD4^+CD25^+$ T cells in response to immunization with Ins. B:9-23 peptide (Mukherjee et al. 2003b).

Insulin B-Chain, Ins. B:9-23 Peptide Inhibit Diabetes Incidence in NOD Mice

As previously reported 4-week-old female NOD/Ltj mice were immunized i.p. or subcutaneously with 100 μg of Ins. B:9-23 peptide (Table 1) emulsified in IFA/saline (Mukherjee et al. 2003b). The Ins. B9-23 peptide inhibited the diabetes incidence by over 80% at 30 weeks of age (Table 2). All the IFA/saline-treated control mice developed diabetes by 30 weeks of age ($p < 0.05$).

Cytokine Analysis of Insulin B-Chain, Ins. B:9-23 Peptide Peptide-Primed NOD T Cell Supernatants

To determine the cytokines elicited by Ins. B:9-23 peptide in NOD mice, culture supernatants of $CD4^+$ T cells from peptide immunized mice were analyzed. Female NOD/Ltj mice aged 6–8 weeks were injected with 100 μg of Ins. B:9-23 peptide emulsified in IFA. Ten days later, T cells were isolated from the draining lymph nodes, stimulated with Ins. B:9-23 peptide for 72 h in vitro. Supernatant from these cell cultures were analyzed by ELISA for various cytokines as shown in Table 2. We found that B:9-23 peptides induced secretion of TGF-β (>500 pg/ml) and TNF-α (200 pg/ml) and only low levels of IL-10, IFN-γ and IL-4 (Table 2). These data support the induction of Th3 cells by Ins. B:9-23 peptide.

Peptides from IGRP Induce TGF-β-Producing Th3 Cells and Th2 Cells that Inhibit Diabetes Development in NOD Mice

IGRP, also known as G6PC2, is a highly conserved protein in humans and rodents (Martin et al. 2001). It is an important autoantigen in T1D (Clemente-Casares et al. 2016; Han et al. 2005a, b; Lieberman et al. 2003; Mukherjee et al. 2005; Serra and Santamaria 2015; Yang et al. 2006). In NOD mice, majority of the diabetogenic $CD8^+$ T cell population recognizes a nine amino acid peptide, IGRP p206-214, in this protein (Lieberman et al. 2003; Wong et al. 2006). We have found spontaneous and primed $CD4^+$ T cell responses to a number of IGRP peptides and have shown that many of these peptides inhibited T1D development in NOD mice (Mukherjee et al. 2005).

IGRP Peptides IGRP p123-145 and IGRP p195-214 Reduce Onset of Diabetes in NOD Mice

As summarized in Table 2, immunization with IGRP p123-145 and IGRP p195-214 peptides (Table 1) provided protection from T1D in NOD mice (Mukherjee et al. 2005). For these studies, 4–5-week-old female NOD/Ltj mice were immunized with 100 μg of IGRP peptides emulsified in IFA/saline. With IGRP p123-145 peptide, 30% of NOD mice developed diabetes while only 10% of mice immunized with IGRP p195-214 developed disease by 30 weeks of age (Table 2) (Mukherjee et al. 2005). Therefore, both of these IGRP peptides significantly reduced the diabetic incidence in NOD mice ($p < 0.05$).

Cytokine Analysis of IGRP p123-145 and IGRP p195-214 Peptide-Primed NOD T Cell Supernatants

Analysis of cytokine production was undertaken to identify the T cell subsets that were induced in response to priming of NOD mice with IGRP peptides. Four to five-week-old female NOD mice were immunized with 100 μg of IGRP peptide emulsified in IFA/saline. After 10 days, $CD4^+$ T cells isolated from the draining lymph nodes were stimulated with the IGRP peptide in tissue culture. After 72 h the

culture supernatants were analyzed for various cytokines by ELISA (Table 2). Cells primed with IGRP p123-145 produced moderate levels (2500 pg/ml) of IFN-γ, significantly high levels (750 pg/ml) of IL-10, modest levels (100 pg/ml) of IL-4 and low levels (20 pg/ml) of TGF-β. Cells induced by IGRP p195-214 also generated moderate levels (1600 pg/ml) of IFN-γ, low levels (100 pg/ml) of IL-10 and IL-4 (<10 pg/ml), but higher levels (850 pg/ml) of TGF-β. Based upon the cytokine profiles, IGRP p123-145 peptide largely induced Th2 cells while IGRP p195-214 peptide induced TGF-β-producing regulatory Th3 cells. IGRP p195-214 peptide that induced TGF-β producing Th3 cells is much more potent in disease prevention.

MHC Class II I-Aβ^{g7} p54-76 Peptide Induce Tr1 Cells that Produce TGF-β and Prevent Diabetes in NOD Mice

We previously reported that immunization of young NOD mice with I-Aβ^{g7} p54-76 peptide derived from self-MHC class II I-Aβ chain prevented development of T1D in NOD mice (Chaturvedi et al. 2000). This was shown to be mediated by regulatory Tr1 cells that produced large amounts of TGF-β (Mukherjee et al. 2003a). Culture supernatant from I-Aβ^{g7} p54-76 peptide-primed CD4$^+$ T cells secreted large amounts of IL-4, IL-10 and TGF-β. A T cell line (I-A.Y) specific for I-Aβ^{g7} p54-76 peptide and generated from NOD mice also secreted IL-4, TGF-β and IL-10 and did not induce diabetes upon adoptive transfer in NOD/SCID mice (Mukherjee et al. 2003a). It has been previously shown by Han et al. (1996) that self-MHC class II reactive suppressor T cells (NY4.2) inhibit the proliferation of effector T cells from NOD mice. These cells prevented spontaneous diabetes and recurrent diabetes in islet transplanted NOD mice. Similar to our I-A.Y T cells, the suppressive activities of the self-MHC class II specific regulatory T cells (NY4.2) were mediated by the secretion of a large amount of TGF-β (Han et al. 1996, 1997).

Peptide Immunotherapy and TGF-β

Our disease protection studies have revealed that the four GAD65 and GAD67 peptides provided varying levels of protection from T1D. The current T1D disease paradigm dictates that Th1 and Th17 cells induce inflammatory responses mediated by IFN-γ and IL-17 cytokines, respectively (Fig. 1). On the other hand, regulatory cytokines such as IL-4, IL-10 and TGF-β produced by immunoregulatory Th2, iTreg, iTreg17, Th3 and Tr1 cells are strongly protective (Fig. 1) (Bach 1994; Delovitch and Singh 1997; Singh et al. 2011, 2013). The induced Treg (iTreg and iTreg17) cells are likely generated in the periphery following peptide immunization and are different from thymus-derived natural Treg cells. It has been reported that immunization of NOD mice with a number of different autoantigens or their immunodominant epitopes results in protection from T1D (Peakman and von Herrath 2010; Zechel et al. 1997). In our studies, the GAD65 and GAD67 peptide-primed CD4$^+$ T cell supernatants tested negative for TNF-α production. Only GAD65 p202-221 and GAD67 p210-229 primed T cells showed low levels (190–200 pg/ml) of IL-10 production. Importantly, all protective GAD peptides resulted in high levels of TGF-β (805–1050 pg/ml) production. This is in line with previous studies that demonstrated that pre-incubation of islets with 1 ng/ml of TGF-β was sufficient to completely inhibit lymphocyte-mediated cell lysis of islet cell monolayers (Koevary 1991). Furthermore, TGF-β possesses a reciprocal relationship with IFN-γ where TGF-β downregulate the pro-inflammatory activity of IFN-γ (Li et al. 2007; Strober et al. 1997).

In addition to its suppressive capabilities, TGF-β has been shown to upregulate Treg cells (Ishigame et al. 2013; Singh et al. 2013; Wan and Flavell 2006) and shift antigen-presenting cells preference (King et al. 1998). A number of Treg cells that modulate autoimmunity have been identified in NOD mice (Chaturvedi et al. 1997; Chen et al. 2003). These include Th3 (Chen et al. 1994) and Tr1 (Groux 2003; Groux et al. 1997) T cell subsets that secrete varying levels of IL-2, TGF-β and IL-10. However, a clear distinction regarding the involvement of these T cell subsets can be made. Tr1 cells have been shown to secrete large quantities of IL-10 and moderate amounts of TGF-β and TNF-α (Cottrez et al. 2000; Groux 2003). The cytokine profiles illustrated here are indicative of recent reports for Tr1 cells (Clemente-Casares et al. 2016). Conversely, Th3 clones that secrete TGF-β^{hi}, IL-4^{lo}, IL-10^{lo} have been identified (Chen et al. 1994). Therefore, it is likely that Th3 regulatory cells are responsible for the immunomodulation observed in our studies. The presence of the moderate level of IFN-γ in our GAD peptide-stimulated culture supernatants can be attributed to the presence of a CD4$^+$ clone that secretes IFN-γ in conjunction with TGF-β and IL-10.

We have previously reported that insulin B-chain peptide B:9-23 induced CD4$^+$CD25$^+$ Treg cells and these T cells prevented adoptive transfer of diabetes (Mukherjee et al. 2003b). These regulatory T cells are likely to be of Th3 subset and similar to cells induced by GAD65 and GAD67 peptides as they secreted large amounts of TGF-β and TNF-α with little or no IFN-γ and IL-10.

IGRP has emerged as an important autoantigen in T1D in NOD mice (Han et al. 2005a, b; Ko et al. 2014; Lieberman et al. 2003; Mukherjee et al. 2005; Yang et al. 2013b). Role of IGRP has also been implicated in human T1D (Yang et al. 2006). We identified several immunogenic peptides of IGRP that are recognized by CD4$^+$ T

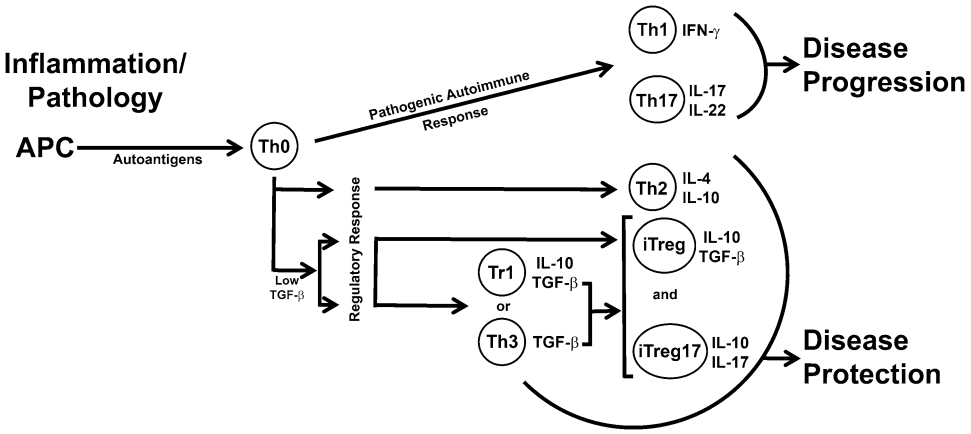

Fig. 1 Modulation of autoimmune diseases by TGF-β. Antigen-presenting cells (APC) drive pathogenic or regulatory immune responses. In a pro-inflammatory environment the naïve Th0 cells differentiate into pathogenic Th1 and Th17. These cells participate in the induction and progression of autoimmune responses. Under non-inflammatory conditions the naïve Th0 cells differentiate into inhibitory Th2 cells or regulatory T cells that inhibit pathogenic Th1 and Th17 cells, including Th3, Tr1, iTreg and iTreg17 cells. These regulatory T cells are non-pathogenic and produce TGF-β in an autocrine fashion. Major cytokines produced by various helper T cells are indicated

cells in the NOD mouse and some of these peptides can modulate disease (Mukherjee et al. 2005). Two of these peptides, IGRP p123-145 and IGRP p195-214, induced Th2 and Th3 Treg cells, respectively. The TGF-β secreted by Th3-type cells inhibits Th1 responses against self'-antigens without compromising the ability to respond to non-self antigen (Li et al. 2006). Th2-type cells are cell contact-dependent and secrete IL-4, which acts globally to downregulate autoimmune responses (Tisch et al. 2001). Also, Treg clones which secrete IFN-γ, IL-10 and TGF-β have been shown to infiltrate the pancreatic islets, similar to the phenomenon observed previously (Han et al. 1996).

We have previously shown that there is a spontaneous breakdown of tolerance to self-MHC class II I-Aβ^{g7} p54-76 peptide in NOD mice with age, but tolerance to this peptide can be maintained by immunizing mice with this peptide at an early age (Chaturvedi et al. 2000). The Treg cells induced by this peptide prevented spontaneous and adoptive transfer of T1D in NOD mice (Mukherjee et al. 2003a). Supernatant from I-Aβ^{g7} p54-76 peptide-primed T cells inhibited the T cell responses in vitro suggesting that the inhibition is mediated by soluble factors secreted by I-Aβ^{g7} p54-76 peptide-primed cells. Cytokine analysis of the supernatant from I-Aβ^{g7} p54-76 peptide-primed cells showed the presence of IL-4 (Chaturvedi et al. 2000), low amounts of IL-10 and high amounts of TGF-β (Mukherjee et al. 2003a). The I-A.Y T cell line mentioned above, and specific for the I-Aβ^{g7} p54-76 peptide was established from peptide-primed NOD mice. In vitro, this line secreted large amounts of TGF-β and IL-10 and in vivo was non-diabetogenic and suppressed the adoptive transfer of diabetes in NOD/SCID mice. The combination of TGF-β and IL-10 production by immunization with I-Aβ^{g7} p54-76 peptide and by the I-A.Y cell line suggested that this response was mediated by Tr1 immunoregulatory cells (Mukherjee et al. 2003a).

A role of TGF-β has been implicated in a wide variety of in vivo models of autoimmunity including in NOD mice making this cytokine an important candidate for T cell-mediated immunoregulation (McCarron and Marie 2014; Richer et al. 2008; Sanjabi et al. 2017; Suarez-Pinzon and Rabinovitch 2001). As outlined above, it also has a major role in immune tolerance in autoimmune diseases such as T1D. A number of studies have reported the secretion of TGF-β by self-MHC-reactive Treg cells that mediate oral and peripheral tolerance and prevent the development of autoimmune diseases (Han et al. 1996; Kitani et al. 2000). Our studies with I-Aβ^{g7} p54-76 peptide in NOD mice discussed above further support these conclusions.

TGF-β signaling controls T helper cell differentiation both in and outside of the thymus. Reduced TGF-β signaling increases peripheral Th1 cells that are involved in the development of T1D in NOD mice. This also increases the level of IFN-γ produced by these Th1 cells. This has been supported by deletion of TGF-β receptor signaling in CD4^{+} BDC2.5 T cells that significantly increased their diabetogenic potential (Ishigame et al. 2013). Loss of TGF-β can also drive Treg cells into Th1 effector cells (Murai et al. 2009; Zhou et al. 2009). The role of TGF-β in maintaining peripheral tolerance is likely to be important in preventing the development and onset of autoimmune diseases such as T1D. In this context, the mechanism of action of TGF-β involves Smad4 as a key signaling molecule to suppress pathogenic T cells and prevent T1D in NOD mice. Smad4-knockout NOD mice were recently shown to have increased incidence of diabetes (Kim et al. 2017). Therefore, lack of TGF-β signaling by Smad4

deletion in T cells of NOD mice dysregulated T cell activation and accelerated T1D. This again confirms the essential role of TGF-β in preventing T1D development (Kim et al. 2017; Zeng et al. 2015).

Conclusion

We conclude that TGF-β is central in regulating autoimmunity through its role in the thymus and periphery. It limits the generation of pro-inflammatory effector T cells and supports the development of anti-inflammatory Treg cells. Immunization with specific autoantigenic self-peptides upregulates TGF-β-producing Treg cells in the periphery, which act to downregulate autoimmunity and reduce or prevent disease.

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