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Context and location dependence of adaptive Foxp3+ regulatory T cell formation during immunopathological conditions

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Abstract

Circulating Foxp3$^+$ regulatory T cells (Treg) may arise in the thymus (natural Treg, nTreg) or through the adaptive upregulation of Foxp3 after T cell activation (induced Treg, iTreg). In this brief review, we explore evidence for the formation and function of iTreg during pathologic conditions. Determining the ontogeny and function of Treg populations has relied on the use of manipulated systems in which either iTreg or nTreg are absent, or lineage tracing of T cell clones through repertoire analyses. iTreg appear particularly important at mucosal interfaces. iTreg can also ameliorate tissue-specific autoimmunity and are a prominent source of tumor-infiltrating Treg in some models. However, under many conditions, including in CNS autoimmunity, diabetes, and some tumor systems, iTreg formation appears limited. The immunological contribution of iTreg is thus highly context dependent. Deciphering immune parameters responsible for iTreg formation and their role in modulating pathologic immune responses will be important.

Keywords

regulatory T lymphocyte; nTreg; iTreg; EAE; diabetes; tumor; colitis; TCR repertoire

1. Introduction

CD4$^+$ regulatory T cells (Treg) that express the forkhead box p3 (Foxp3) transcription factor are essential for immune regulation[1]. Treg depletion, or absent or dysfunctional Foxp3 leads to fulminant, multi-organ autoimmunity and early death[2]. Tregs have a unique capacity to suppress the immune response, and operate through many mechanisms, including expression of inhibitory cell surface proteins such as CTLA-4, secretion of suppressive cytokines such as IL-10 and TGF-β, metabolic disruption, and direct cytolysis [3–5].

Treg can arise both from developing thymocytes, a population referred to as natural Treg (nTreg), or from activated conventional T cells (Tconv) that upregulate Foxp3 in the periphery, referred to as adaptive or induced Treg (iTreg). Foxp3 is induced in naive T cells after TCR stimulation in the presence of IL-2 and TGF-β [6, 7]. This is modulated by a variety of signaling pathways that impinge directly or indirectly on the Foxp3 promoter,

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(Table 1)[8, 9]. STAT5 is phosphorylated and translocates to the nucleus with IL2 stimulation, RUNX1/3 and other factors with the TCR, and Smad3 with TGF-β [10–16]. These bind Foxp3 promoter and enhancer elements to support Foxp3 transcription. Other transcription factors such as STAT3, activated in response to inflammatory cytokines including IL-6, IL-21, and IL-23, are repressive[17–21]. Interestingly, some of the same inflammatory signals that inhibit Foxp3 expression and hence Treg sustenance, are also necessary for Treg function. STAT3 is required for Treg suppression of Th17-mediated immunity. Treg similarly utilize Tbet for Th1, IRF4 for Th2, and Bcl6 for Tfh suppression[2, 8, 9]. Thus Treg formation, persistence, and activity exist in a dynamic balance established by local inflammatory and homeostatic inputs.

In vivo conditions that promote iTreg formation are not well resolved, though homeostatic expansion of T cells in lymphopenic conditions, provision or chronic exposure to low dose or oral antigen in non-inflammatory conditions, and antigen presentation by immature DCs are favorable[22–27]. Blocking studies indicate an *in vivo* role for IL-2 and TGF-β in iTreg induction[28–30]. Retinoic acid (RA) fosters iTreg formation in part by enhancing TGF-β production[31–33]. Additional pathways, including aryl hydrocarbon receptor signals and mTor inhibition further promote Foxp3 upregulation (Figure 1)[34–37]. Once induced, Foxp3 binds its own promoter, helping stabilize its own expression while also inhibiting effector T cell differentiation by, for example, antagonizing RORγt function[9, 38]. Foxp3 promoter elements are not demethylated in iTreg to the extent that they are in nTreg, and iTreg show significant instability[39, 40]. Indeed, though generally suppressive, in pathologic conditions, iTreg can revert to effector forms that contribute to immunopathology[22, 41].

nTreg and iTreg share many regulatory properties, but are non-redundant[42, 43]. This in part reflects the distinct TCR repertoires of iTreg and nTreg. Whereas nTreg are derived from thymocytes selected for self-recognition, iTreg develop from conventional T cells and are more likely to recognize foreign antigens. This would be expected to guide these cells to different targets [44–46]. Their functional activities may also differ, and iTreg and nTreg have distinct gene expression profiles[47]. The formation and relative role of iTreg and nTreg within disease states is not well understood. Because phenotypic markers that definitively distinguish iTreg and nTreg are lacking, analysis of each population's presence and activity during disease has presented significant technical challenges. Here, we review data from several model systems exploring the generation, stability, and function of iTreg during pathologic conditions.

2. iTreg formation in gastrointestinal (GI) immunity

The GI mucosa interfaces with commensal microbiota and ingested food. Immune reactions to microbial and food antigens can lead to inflammatory bowel disease (IBD) or food intolerances. nTreg are positively selected in the thymus for self antigen recognition. As ingested and microbial antigens will not be expressed in the thymus, selection of nTreg specific for these will be limited[24]. Maturation of gut antigen-specific T cells into iTreg may therefore be important to preserve GI homeostasis. Indeed, iTreg specific for both microbes and fed antigens are found in the GI tract. These iTreg provide protection against colitis in a manner that complements rather than duplicates the effects of nTreg[42, 43, 48].

Mice deficient in the conserved nucleotide sequence 1 (CNS1) in the Foxp3 gene do not develop iTreg. CNS1 includes a TGF-β-NFAT response element and is required for iTreg but not nTreg formation. Systemic autoimmunity is not seen, but CNS-1 deficient mice are susceptible to spontaneous colitis[49]. Several GI-specific mechanisms may be involved. RA-induces iTreg that upregulate the gut homing receptors α4β7 integrin and CCR9, and

can be found in the lamina propria[33, 50, 51]. $CD103^+$ dendritic cells in the mesenteric lymph node (MLN) promote tolerance through the generation of iTreg in the context of TGF-β and RA [52, 53]. Interestingly, tolerance generated in the gut can also inhibit the progression of distant autoimmune diseases, including diabetes and experimental allergic encephalomyelitis (EAE)[24, 49, 54].

3. iTreg formation in central nervous system autoimmunity

Treg accumulate in the CNS of mice with EAE, and can comprise 20–40% of infiltrating $CD4^+$ T cells late in disease. Transfer of iTreg or nTreg into mice inhibits EAE, whereas Treg depletion worsens disease[55]. *In vitro*, neurons stimulated the conversion of encephalitogenic T cells into iTreg in a B7 and TGF-β dependent manner[56]. Further, cells from nTreg-deficient $Rag^{-/-}$ mice transgenic for a myelin basic protein-specific TCR were able to differentiate into iTreg during homeostatic expansion[57]. This suggests that myelin specific iTreg may form *in situ*.

To assess the origin of CNS infiltrating Treg in mice with a heterogeneous T cell repertoire, we monitored conventional and regulatory TCR sequences in the CNS and spleen of wild type mice or mice with a fixed TCRα chain during EAE using high-throughput sequencing. CNS CDR3 sequences from Treg and Tconv showed little commonality, but overlapped with the corresponding Treg or Tconv sequences in the spleen[58, 59]. Further, in the mice with a fixed TCRα, analysis of TCRβ sequences bearing defined Vβ and Jβ identified specific amino acids in the CDR3β of myelin-reactive receptors that were specifically and alternatively associated with Treg or Tconv lineage assignment. This indicates a distinct ontogeny for the populations, and argues against substantial iTreg formation in the setting of a diverse TCR repertoire[58]. Consistently, little iTreg formation was detected among $Foxp3^-$ T cells adoptively transferred into mice prior to EAE induction[60]. CNS1-deficiency, which impedes iTreg formation, also did not affect EAE severity, implying that iTreg are not required for EAE protection [49].

4. iTreg formation during diabetes

iTreg can be induced in NOD mice after immunization with insulin, and Treg transfer is able to block disease [61]. The involvement of iTreg in spontaneous diabetes in NOD mice has not been established. All trans retinoic acid (ATRA) increased Treg number and protected mice from insulitis[62]. Depletion of Treg prior to ATRA treatment abrogated protection, suggesting that ATRA-induced iTreg that may have formed were not sufficient to confer protection. In a model targeting a proinsulin mimiotope to DCs in NOD mice, iTreg also formed. These did not impact disease progression, but appeared to reduce the incidence of diabetes[63].

More data is available in the BDC2.5 TCR transgenic model of diabetes[63–65]. In a $RAG^{-/-}$ T cell transfer model anti-thymocyte globulin (mATG) led to immune tolerance that was dependent on the induction of $CTLA4^+$ BDC2.5 iTreg effectors, indicating that in specific therapeutic contexts iTreg formation can be relevant[66, 67]. T cells in TCR transgenic models often express a second endogenously rearranged TCRα chain. Analysis of the repertoire of these second chains in BDC2.5 mice with diabetes indicated that islet-infiltrating Treg are unrelated to Tconv. This indicates that iTreg do not normally contribute significantly to the Treg response during diabetes in these mice[68].

5. iTreg formation in the tumor microenvironment

Tumors can create an immunosuppressive environment that may promote iTreg development through TGF-β production or other mechanisms[69]. Indeed, many tumors

show large numbers of infiltrating Treg[70]. The extent to which these result from the induction of Foxp3 in conventional T cells or nTreg recruitment appears to be tumor dependent. In MO-5 (B16-ova) and TC-1 tumor models, repertoire analyses indicated that Treg infiltration was the result of homing and expansion of circulating Treg, and not intratumoral iTreg formation[71]. $Foxp3^+$ iTreg also could not be recovered from OT-II mice implanted with TGF-β-producing B16-ova tumors, and Treg infiltrates in a murine glioblastoma (GBM) appeared to be predominantly of thymic origin[72, 73]. Repertoire analysis of carcinogen induced tumor-infiltrating Tconv and Treg displayed minimal TCR overlap. Greater similarity was observed between tumor infiltrating cells and those in the tumor draining lymph nodes, arguing against significant conversion[70].

In contrast with these reports, a separate study of B16F1 melanomas indicated substantial overlap between the tumor-responsive Tconv and Treg repertoires, implying that iTreg induction supplied a large component of the Treg response. A similar high level of repertoire overlap was seen in Treg isolated from patients with melanoma [74, 75]. Cumulatively, these data indicate tumor-specific variation in the origins of infiltrating Treg, with only some tumors showing substantial iTreg formation. Deciphering the environmental cues guiding the intratumoral induction of iTreg will be important to optimally counteract Treg suppression of the tumor-specific immune response.

6. Conclusions

Formation of iTreg during immunopathologic conditions shows regional and contextual variability. iTreg formation has been clearly identified within the GI tract. There is a strong rationale for iTreg generation at mucosal and integumentary interfaces based on specificity considerations. Continuous mucosal exposure to large quantities of predominantly innocuous environmental antigens leads to a strong risk for immunopathology due to overzealous immune responses. Diversion of reactive T cells to iTreg may help quell such responses.

In contrast with mucosal immunity, nTreg, which develop in response to thymic self antigens, will possess a repertoire biased toward recognition of a host's own tissues, one that should be capable of limiting autoreactive responses. The absence of robust markers that distinguish nTreg and iTreg prevents ontologic categorization of Treg by direct staining. Nevertheless, we and others have used indirect approaches and have not found a significant contribution of iTreg during some organ specific autoimmune responses where a heterogeneous TCR repertoire is present. Likewise, in some but not all studies, nTreg are the primary source for tumor-infiltrating Treg.

While it is clear that adaptive upregulation of Foxp3 on T cells occurs, it remains to be determined why this is variably supported in different anatomic locations and environmental conditions. Possibly, the small number of iTreg that do form during autoimmune diseases expand to fill a niche made available by the absence of nTreg. Both iTreg and nTreg will be supported by the same tropic cytokines, and competition between these populations is possible. Alternatively or in a complementary manner, iTreg, which arise from Tconv with a broad specificity for foreign antigens, may fill specificity gaps left in the nTreg repertoire, which is skewed toward self-specificity. A better understanding of the signals that induce and sustain iTreg will be critical for the development of enhanced therapeutics capable of manipulating these essential regulators of the immune system.

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Abbreviations

Foxp3	forkhead box p3 transcription factor
Treg	regulatory T lymphocyte
nTreg	natural Treg
iTreg	induced Treg
Tconv	conventional T cell
TCR	T cell receptor
EAE	experimental allergic encephalomyelitis
CNS	central nervous system
CDR	complementarity determining region.

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Highlights

- iTreg and nTreg are non-redundant, and have distinct roles and antigen specificity.
- iTreg are important for the induction of gastrointestinal tolerance.
- iTreg formation is inconsistent in models of autoimmune or neoplastic disease.
- The role of iTreg in immunopatholgic conditions is context and location dependent.

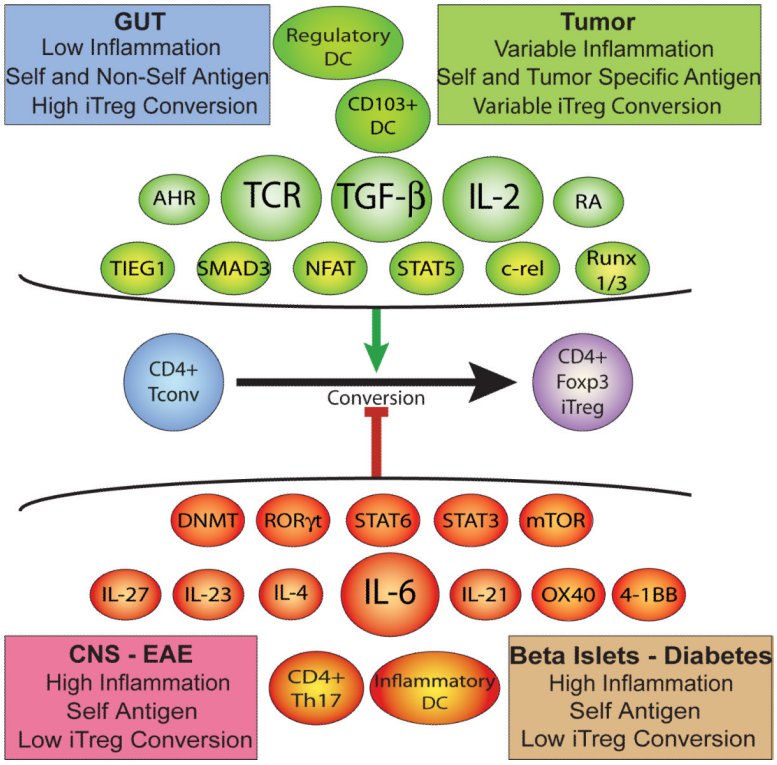

Figure 1.

Foxp3 induction and iTreg formation is regulated by different antigen presenting cell types, cytokines and cell signaling molecules, signaling pathways, and transcriptions factors. The integration of positive and negative inductive signals will determine whether Foxp3 is upregulated. The distinct environments of the gastrointestinal tract, CNS, islets, and tumors will variably promote the formation of iTreg.

Table 1

Molecules and stimuli positively or negatively regulating iTreg development are listed.

Positive Regulators	Citation	Negative Regulators	Citation
IL-2	[76–78]	IL-6	[18, 19]
TGF-β	[79–82]	IL-4	[83, 84]
TCR	[85–87]	IL-21	[19, 20]
Low Dose or Chronic Antigen Exposure	[25, 88–90]	IL-23	[21]
IL10	[91, 92]	IL-27	[17, 93]
RA	[31–33, 89, 94, 95]	TNFα	[96, 97]
AHR	[34, 35, 43]	OX40	[96, 97]
Vitamin D3	[98]	mTor, S1P1, PI3K	[99, 100]
Rapamycin	[6, 36, 37, 78, 101]	PCKθ	[102]
Foxp3	[1, 5, 18, 22, 103, 104]	4-1BB	[105, 106]
RORγt	[18, 107]	CD28 (Strong)	[108]
Smad3	[10–12, 109]	RORγt	[18, 19]
Smad4	[13, 109]	STAT3	[17]
STAT5	[14, 15]	STAT6	[32]
STAT1*	[110]	NOTCH/Hes1*	[111–113]
Id3	[114]	DNMT	[40, 115]
E2a	[114]	GATA3*	[114, 116]
Runx1/3	[16]		
c-rel	[117, 118]		
NFAT	[12, 104, 118, 119]		
AP-1	[9, 120]		
CREB	[39]		
FoxO1/O3a	[102, 121]		
Sp-1	[40]		
TIEG1	[40, 122]		

* Both positive and negative regulation documented.