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Comprehensive phenotyping of mouse regulatory T cells relevant to viral infections

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Abstract

Regulatory T (Treg) cells are a specialized subpopulation of $CD4^+$ T cells that enforce peripheral immune tolerance. Treg cells act to suppress exuberant immune responses, limit inflammation, and promote tissue repair, thereby maintaining homeostasis and tolerance to self-antigens and those of the commensal microbial flora. Treg cells are characterized by the expression of the master regulator Foxp3, which plays a major role in Treg cells development and function. Under inflammatory conditions, $Foxp3^+$ Treg cells may acquire effector T cell programs that modify their phenotype and function, reflecting their plasticity. During microbial infections, Treg cells act to limit the immunopathology triggered by the host immune response to pathogens albeit at the potential risk of pathogen persistence. In this review, we will discuss the influence of Treg cells on the outcome of viral infection and will give an overview of the Treg phenotype at steady-state and in inflammatory conditions.

KEYWORDS
flow cytometry, phenotyping, regulatory t cells, viral infection

1 | INTRODUCTION

Regulatory T (Treg) cells expressing the intracellular transcription factor forkhead box P3 (Foxp3) play a requisite role in the maintenance of immunological homeostasis and prevention of self-tolerance breakdown [1]. In 1995, Sakaguchi first described a unique population of $CD4^+$ T lymphocytes that express the IL-2Rα chain (CD25) and suppress autoimmune disease in thymectomized mice and autoimmunity mice models [2]. Further studies revealed that these $CD25^+CD4^+$ T cells express Foxp3 [3, 4]. Mutation or deletion of Foxp3 induces a lethal autoimmune syndrome called IPEX (immune dysregulation polyendocrinopathy enteropathy X-linked syndrome) in human [3, 4]. Treg cells can be identified by the combination of different surface markers and the key lineage-defining transcription factor Foxp3 [5, 6]. They ensure peripheral immune tolerance by controlling autoreactive T cells that have escaped from thymic selection and their loss results in autoimmune disease [7, 8]. They are a critical key player for self-tolerance establishment and control of pathology in various inflammatory responses to mediate immune homeostasis [9]. Treg cells accumulate at the inflammation site, most likely to control the magnitude and the excessive of the inflammation. They are essential for suppressing pro-inflammatory immune responses and protecting the host from immune-mediated pathology [10–14]. Treg cells integrate various environmental conditions to coordinate and adapt their effector activities in the sites of inflammation. In addition, Treg cells are considered to play a crucial role in several mouse infection disease models [15, 16].

1.1 | Treg cell maker expression and subsets

Treg cells can be divided into multiple sub-populations defined by their origin, the expression of cellular markers, and their mechanisms of function. They represent 5%–15% of total $CD4^+$ T cells and express αβ T cell receptors (TCR) [17]. Unlike human Treg cells, mouse Treg cells

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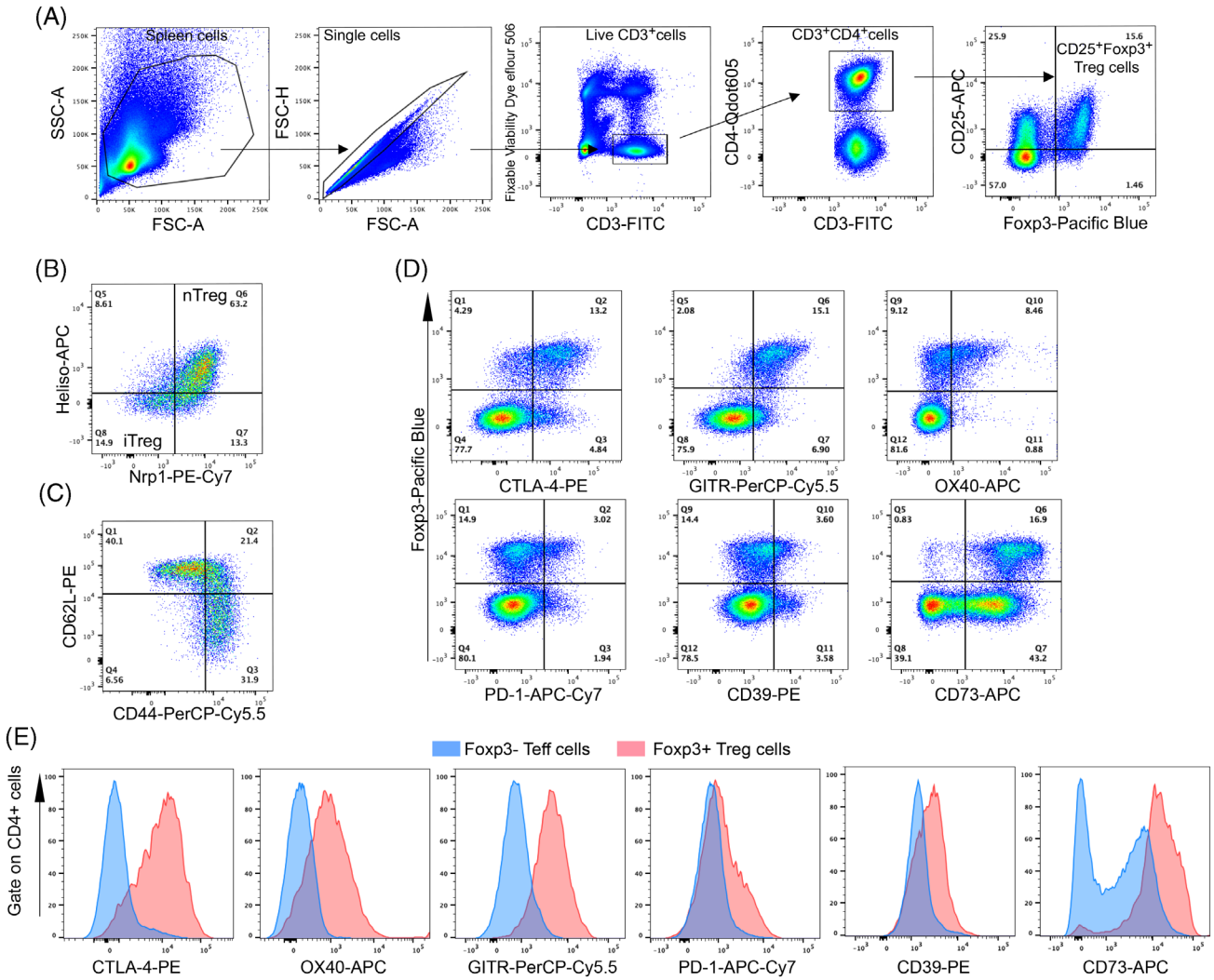

FIGURE 1 Flow cytometric detection of Treg cells population in mice spleen tissue (A) gating strategy of splenic Treg cells, Treg cells are express CD3+ CD4+ CD25+ Foxp3+. (B) Define nTreg and iTreg population-based on Helios and Nrp-1 expression. nTreg (Helios^{+} Nrp-1^{+}) and iTreg (Helios^{-} Nrp-1^{-}). (C). Determine the naïve Treg and activated Treg cells by CD62L and CD44 marker expression. Naïve Treg (CD44^{lo}CD62L$^{hi)}$ and activated Treg (CD44^{hi}CD62L^{lo}). (D) co-stimulatory molecules expression on Foxp3+ Treg cells and Foxp3- effector T cells (Teff). (E) Overlaid shaded histograms of T cell-specific co-stimulatory markers expression on Foxp3^{+} Treg cells and Foxp3^{-} Teff cells. [Color figure can be viewed at wileyonlinelibrary.com]

represent a more homogeneous population and are characterized by the expression of CD4, CD25, and the intracellular transcription factor Foxp3. They have been also revealed to express several co-stimulatory molecules such as CD28, CD40L, cytotoxic T lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced TNF receptor (GITR), programmed cell death-1 (PD-1) and OX40 (CD134) [18]. CD28 and CD40L play a role in the maintenance of peripheral Treg homeostasis, whereas CTLA-4, GITR, PD-1, and OX40 are involved in the regulation of immunosuppressive activity [19–23]. Apart from that, Treg cells isolated from secondary lymphoid organs express the spleen/lymph node homing receptors CCR7 and CD62L [24]. In addition, CD39 is constitutively expressed on murine Tregs, which are closely associated with CD73, where it plays a central role in mediating the immunosuppression [25]. Treg cells also produce immunosuppressive cytokines including TGF-β, IL-10, and IL-35 [26]. According to their origin, Treg cell populations can be divided into two main groups: natural Treg cells (nTreg cells) which develop in the thymus, and induced Treg cells (iTreg cells) which develop in the periphery from naïve conventional T cells [27–30]. Both nTreg cells and iTreg subsets have their roles in the immune system. The nTreg cells are mainly auto-specific and control autoimmune response, while iTreg cells develop in response to externally delivered antigens or commensal microbes. Helios, a member of the Ikaros transcription factor family is highly expressed in nTreg cells, but not in iTreg cells [27]. Similarly, neuropilin-1 (Nrp-1) also serves as an additional marker to distinguish between nTreg and iTreg. Indeed, Nrp-1 is highly expressed on nTreg cells but not on iTreg at steady-state [29, 30]. However, some studies have shown that iTreg cells in the context of inflammatory conditions can also express Nrp1 [30]. To conclude, Treg cells are a heterogeneous population, and a combination of surface and intracellular markers is mandatory to characterize these different subpopulations.

TABLE 1 Markers expressed on CD4+ T cells subset

	nTreg	iTreg	Naïve Tregs	Activated Treg	Naïve T effector cells	Activated T effector cells
CD3	+	+	+	+	+	+
CD4	+	+	+	+	+	+
CD25	+++	++	+++	+++	+	++
CD127	low	low	low	low	+	++
Foxp3	++	+	+	++	−	−
Helios	++	−	+	++	−	−
Nrp1	+	−	+	++	−	−
OX40	++	++	++	++	+/−	++
LAP	++	++	++	++	+/−	+
CD69	+	++	+	++	+	++
TIGIT	++	++	++	++	+/−	++
ICOS	++	++	++	++	low	High
CD44	low	high	low	high	low	high
CD62L	high	low	high	low	high	low
CTLA-4	++	+++	+	+++	+/−	++
GITR	++	+++	++	+++	+/−	++
Lag3+	++	++	++	++	+/−	++
PD-1	++	++	++	++	+/−	++
CD39	++	++	++	++	+/−	++
CD73	++	+++	++	+++	+/−	++

1.2 | Characterization of mouse Treg cells by flow cytometry

Flow cytometric analysis with a combination of specific surface and intracellular Treg cells markers helps to characterize Treg cells (Figure 1, Table 1). These analyses should be always preceded by a proper gating strategy, using a single cell gate, and identifying dead cells with a live/dead stain. Within the lymphocytes gate, Treg cells are defined as the $CD3^{+-}$ $CD4^{+}CD25^{+}Foxp3^{+}$ cell population (Figure 1A). This population can be further characterized as nTreg ($Helios^{+}Nrp1+$) and iTreg ($Helios^{-}Nrp1^{-}$) (Figure 1B). Treg cells can be also subdivided into $CD44^{lo}CD62L^{hi}$ 'naive' Treg cells and $CD44^{hi}CD62L^{lo}$ 'activated' Treg cells (Figure 1C). These activated Treg cells express higher levels of Treg effector molecules, such as cytotoxic T cell antigen 4 (CTLA4) compared to 'naïve' Treg cells, which likely contribute to increased their suppressive activity [31]. Some co-stimulatory/co-inhibitory molecules including CTLA-4, GITR, OX40, and CD73, which are crucial for the Treg cell's suppressive function, are highly expressed on Treg cells compared to Teff cells. (Figure 1D, E, Table 1). To conclude a combination of several markers can be used to better identify different functional subsets of Treg cells (Table 1).

1.3 | The role of Treg cells during pathogen infection

Pathogen infections have been suggested as triggers of autoimmunity and several autoimmune diseases show a strong correlation to certain viral infections. Treg cells can directly modulate host immune responses to microbial infections. These Treg cells have both beneficial and adverse effects on disease outcomes during viral infections (Table 2) [32–34]. Several of these discoveries were made using animal models. Indeed, animal models make a major contribution to unraveling the host-pathogen interactions and physiopathology of infections and to better understanding the mechanism of action of those infections. During chronic viral infection, such as lymphocytic choriomeningitis virus, hepatitis C virus (HCV), hepatitis B virus (HBV), and Herpes simplex viruses, these persistence-inducing viruses can trigger massive proliferation of Treg cells that suppress effector T cell proliferation, inflammatory cytokine production and CD8+ T cells cytotoxicity functions [35–39]. In chronic HCV infection, CD25+ Foxp3+ Treg cells contribute to limiting viral-specific CD4+ and CD8+ T cell antiviral immune response, which are important for viral clearance and thereby promoting pathogens persistence [39–41]. Simultaneously, Treg activity could be beneficial to the host due to a suppression of tissue damage mediated by virus-specific effector T cells. In chronic HIV infection, Tregs play both beneficial and detrimental roles. They modulate the immune system and limit the spread of the virus through the suppression of activated T cells, which were targeted by HIV. However, suppression of immune activation also reduces the viral clearance and promotes viral reservoir formation [42, 43]. This Treg-mediated suppression of HIV replication may base on IL-2 dependent Treg cells expansion or cell–cell contact suppression mechanism. Similarly, depletion of Treg cells in a retrovirus infection mouse model prevents murine acquired immunodeficiency syndrome

TABLE 2 The role of regulatory cells during chronic and acute infection

Virus strain	Function of Treg cells during chronic infection	Reference PMID
HCV	CD25+ FOXP3+ Treg population suppress HCV core-specific CD4+ and CD8+ T-cell immune responses	**23,710,581**
LCMV	Absence of IFNAR signaling in Tregs results in decreased $CD8^+$ effector T cells, enhance T effector cell exhaustion, defective generation of antiviral memory $CD8^+$ T cells, and enhanced LCMV persistence	29,672,594 24,711,580
HIV	Treg cells control HIV replication in activated T cells through a cAMP dependent mechanism	**21,436,067**
HBV	Selective depletion of Tregs significantly increase HBV-specific CD8+ T cell responses and accelerate viral antigen clearance The imbalance of Treg/Th17 may link to HBV disease progression	**26,986,976** **22,548,790**
Murine AIDS	Timed ablation of Treg cells can prevent murine AIDS progression	**15,067,071**
HSV-1	CD25+ Treg cells play a beneficial role to minimize viral immunological lesions HSV infection results in increased Treg cells function with such cells able to suppress CD8+ T cell responses to both viral and unrelated antigens Treg cells play a critical role in both HSV-1 latency and reactivation via suppressing anti-viral CD8+ T cells	**12,975,455** **15,034,024** **30,485,807**
HSV-2	Tregs facilitate early protective responses to local viral infection by allowing a timely entry of immune cells into infected tissue CTLA-4 expression by Tregs is critical to promote proper DC migration from the infected tissues and initiating an appropriate antigen-specific CD4 T-cell response	18,436,744 **27,007,674**
Friend Retrovirus	CD4 + CD25+ regulatory T cells suppress CD8+ T cells function and contribute to viral persistence	**16,517,701** **16,981,182**
Vaccinia virus	Treg cells influence CD8+ T cells immunodominance hierarchies	**15,749,866**
Virus strain	**Function of Treg cells during acute infection**	**Reference**
Influenza A virus (IAV)	Treg cells are critical for influenza A virus clearance in neonatal mice Treg depletion enhances CD8 T cells responses to influenza A virus infection Notch4 signaling limits regulatory T-cell-mediated tissue repair and promotes severe lung inflammation after H1N1 infection	**26,501,792** **15,749,866** **33,915,108**
Respiratory syncytial virus (RSV)	Neonatal RSV A2 infection rapidly induces Treg cells suppressive functions to dampen the Th2 and Tc2 responses Treg cells expressing granzyme B can control lung inflammation during acute infection RSV vaccination attenuates airway Treg cells responses to RSV Infection	**33,909,707** 2,223,699 2,338,220
West Nile virus (WNV)	Tregs control the development of symptomatic West Nile virus infection	**19,855,131**

(MAIDS) [44]. Moreover, the Treg/Th17 balance play important role in the occurrence, progression, and outcome of chronic HBV infection [45, 46]. All these studies demonstrated the role of Treg cells in the delicate balance between viral clearance and disease severity. For acute viral infection, most of the studies have focused on the role of Treg cells in influenza virus infection, where lesions are principally the consequence of direct effects of infection. During influenza virus infection, Treg cells depletion through treatment with anti-CD25 mAb significantly enhances CD8+ T cell responses to influenza A virus [47]. It's also has been shown that functional depletion of Treg cells resulted in increased proportions of activated CD4+ T cells in the infected lung tissue, but failure to clear influenza virus [48]. Distinct from their role in suppressing immunity and inflammation, Treg cells promote lung tissue repair and tissue permeability through amphiregulin production [49, 50]. Moreover, Treg cells upregulate Notch4 expression during influenza and SARS-CoV2 infection which was shown to promote lung inflammation due to an inhibition of amphiregulin production [49, 50]. By dampening effector immune responses, Treg cells mitigate immunopathology resulting from exaggerated inflammation and tissue destruction during acute [51–54], or chronic infections ([55], [56–59]). In addition, Treg cells have been shown to support antiviral immunity by modulating T cell migration to the site of infection [53, 60]. Understanding the role of Treg cells in acute and chronic viral infections may provide targets for new therapeutic approaches that will limit tissue damage while maintaining immunity to these pathogens.

1.4 | Tricks and tips for the best characterization of Treg cells

Characterization of mouse regulatory T cells presents some challenges. In this paragraph we will present the tricks and tips for the best Treg cell characterization (see MiFlocyt):

- The first step is to smash and filter the spleen in a 70 mm filter to be able to prepare a cell suspension in RPMI.
- Red blood cells lysis is mandatory for Treg cell characterization.

- Surface staining should be done at 20x10^6/ml in PBS/0.5%FCS.
- Cells should be fixed and permeabilized using eBioscience Fixation/Permeabilization for 30 min at 4°C.
- Intracellular staining should be done at 20 × 10^6/ml in eBioscience Permeabilization buffer.
- Washing after each step is mandatory.
- Use single fluorochrome stained cells as control for compensation of spillover from one channel to another.

AUTHOR CONTRIBUTIONS

Qlan Chen: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal). **Mehdi Benamar:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **TszMan Fion Chan:** Formal analysis (supporting); methodology (supporting). **Muyun Wang:** Formal analysis (supporting); methodology (supporting). **Talal A Chatila:** Conceptualization (equal); funding acquisition (lead); investigation (equal); project administration (lead); resources (lead); supervision (lead); writing – review and editing (lead).

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data of this study are openly available in Flow repository at http://flowrepository.org/id/FR-FCM-Z5YQ

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REFERENCES

1. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133:775–87.
2. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155:1151–64.
3. Malek TR, Yu A, Vincek V, Scibelli P, Kong L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. Immunity. 2002;17:167–78.
4. Furtado GC, Curotto de Lafaille MA, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. J Exp Med. 2002;196:851–7.
5. Fu W, Ergun A, Lu T, Hill JA, Haxhinasto S, Fassett MS, et al. A multiply redundant genetic switch 'locks in' the transcriptional signature of regulatory T cells. Nat Immunol. 2012;13:972–80.
6. Rudra D, DeRoos P, Chaudhry A, Niec RE, Arvey A, Samstein RM, et al. Transcription factor Foxp3 and its protein partners form a complex regulatory network. Nat Immunol. 2012;13:1010–9.
7. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003;4:330–6.
8. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27:20–1.
9. Bin Dhuban K, Kornete M, Mason ES, Piccirillo CA. Functional dynamics of Foxp3(+) regulatory T cells in mice and humans. Immunol Rev. 2014;259:140–58.
10. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. Immunity. 2008;29:114–26.
11. Fyhrquist N, Lehtimaki S, Lahl K, Savinko T, Lappetelainen AM, et al. Foxp3+ cells control Th2 responses in a murine model of atopic dermatitis. J Invest Dermatol. 2012;132:1672–80.
12. Lehtimaki S, Savinko T, Lahl K, Sparwasser T, Wolff H, et al. The temporal and spatial dynamics of Foxp3+ Treg cell-mediated suppression during contact hypersensitivity responses in a murine model. J Invest Dermatol. 2012;132:2744–51.
13. Mason CM, Porretta E, Zhang P, Nelson S. CD4+ CD25+ transforming growth factor-beta-producing T cells are present in the lung in murine tuberculosis and may regulate the host inflammatory response. Clin Exp Immunol. 2007;148:537–45.
14. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y, et al. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. J Clin Invest. 2010;120:883–93.
15. Boer MC, Joosten SA, Ottenhoff TH. Regulatory T-cells at the Interface between human host and pathogens in infectious diseases and vaccination. Front Immunol. 2015;6:217.
16. Veiga-Parga T, Sehrawat S, Rouse BT. Role of regulatory T cells during virus infection. Immunol Rev. 2013;255:182–96.
17. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity. 2009;30:626–35.
18. Tazuma S, Hatsushika S, Aihara N, Yamashita G, Sasaki H, Sagawa H, et al. Effects of a competitive inhibitor of hydroxymethylglutaryl coenzyme a reductase on cholesterol gallstone formation in prairie dogs. Nihon Shokakibyo Gakkai Zasshi. 1991;88:1623.
19. Jain N, Nguyen H, Chambers C, Kang J. Dual function of CTLA-4 in regulatory T cells and conventional T cells to prevent multiorgan autoimmunity. Proc Natl Acad Sci U S A. 2010;107:1524–8.
20. Shevach EM, Stephens GL. The GITR-GITRL interaction: co-stimulation or contrasuppression of regulatory activity? Nat Rev Immunol. 2006;6:613–8.
21. Kitamura N, Murata S, Ueki T, Mekata E, Reilly RT, Jaffee EM, et al. OX40 costimulation can abrogate Foxp3+ regulatory T cell-mediated suppression of antitumor immunity. Int J Cancer. 2009;125:630–8.
22. Zhang X, Xiao X, Lan P, Li J, Dou Y, Chen W, et al. OX40 Costimulation inhibits Foxp3 expression and Treg induction via BATF3-dependent and independent mechanisms. Cell Rep. 2018;24:607–18.
23. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1 (+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. Proc Natl Acad Sci U S A. 2019;116:9999–10008.
24. Wei S, Kryczek I, Zou W. Regulatory T-cell compartmentalization and trafficking. Blood. 2006;108:426–31.
25. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204:1257–65.
26. Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G. Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity. Front Immunol. 2019;10:43.

27. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol. 2010;184:3433–41.
28. Kim YC, Bhairavabhotla R, Yoon J, Golding A, Thornton AM, Tran DQ, et al. Oligodeoxynucleotides stabilize Helios-expressing Foxp3+ human T regulatory cells during in vitro expansion. Blood. 2012;119:2810–8.
29. Yadav M, Louvet C, Davini D, Gardner JM, Martinez-Llordella M, Bailey-Bucktrout S, et al. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. J Exp Med. 2012;209(1713–22):S1–19.
30. Weiss JM, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, Parkhurst CN, et al. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. J Exp Med. 2012;209(1723–42):S1–1742.
31. Yuan X, Cheng G, Malek TR. The importance of regulatory T-cell heterogeneity in maintaining self-tolerance. Immunol Rev. 2014;259:103–14.
32. van der Burg SH, Piersma SJ, de Jong A, van der Hulst JM, Kwappenberg KM, et al. Association of cervical cancer with the presence of CD4+ regulatory T cells specific for human papillomavirus antigens. Proc Natl Acad Sci U S A. 2007;104:12087–92.
33. Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol. 2004;78:2454–9.
34. Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, et al. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology. 2004;40:1062–71.
35. Gangaplara A, Martens C, Dahlstrom E, Metidji A, Gokhale AS, Glass DD, et al. Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. PLoS Pathog. 2018;14:e1006985.
36. Srivastava S, Koch MA, Pepper M, Campbell DJ. Type I interferons directly inhibit regulatory T cells to allow optimal antiviral T cell responses during acute LCMV infection. J Exp Med. 2014;211:961–74.
37. Dietze KK, Schimmer S, Kretzmer F, Wang J, Lin Y, Huang X, et al. Characterization of the Treg response in the hepatitis B virus hydrodynamic injection mouse model. PLoS One. 2016;11:e0151717.
38. Fernandez MA, Yu U, Zhang G, White R, Sparwasser T, Alexander SI, et al. Treg depletion attenuates the severity of skin disease from ganglionic spread after HSV-2 flank infection. Virology. 2013;447:9–20.
39. Ortiz V, Wands JR. Chronic ethanol diet increases regulatory T-cell activity and inhibits hepatitis C virus core-specific cellular immune responses in mice. Hepatol Res. 2014;44:788–97.
40. Losikoff PT, Self AA, Gregory SH. Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C. Virulence. 2012;3:610–20.
41. Self AA, Losikoff PT, Gregory SH. Divergent contributions of regulatory T cells to the pathogenesis of chronic hepatitis C. Hum Vaccin Immunother. 2013;9:1569–76.
42. Kleinman AJ, Sivanandham R, Pandrea I, Chougnet CA, Apetrei C. Regulatory T cells as potential targets for HIV cure research. Front Immunol. 2018;9:734.
43. Moreno-Fernandez ME, Rueda CM, Rusie LK, Chougnet CA. Regulatory T cells control HIV replication in activated T cells through a cAMP-dependent mechanism. Blood. 2011;117:5372–80.
44. Beilharz MW, Sammels LM, Paun A, Shaw K, van Eeden P, Watson MW, et al. Timed ablation of regulatory CD4+ T cells can prevent murine AIDS progression. J Immunol. 2004;172:4917–25.
45. Tan NH, Chen B, Peng J, Du S. Treg/Th17 cell balance in patients with hepatitis B virus-related acute-on-chronic liver failure at different disease stages. Biomed Res Int. 2021;2021:9140602–13.
46. Xue-Song L, Cheng-Zhong L, Ying Z, Mo-Bin W. Changes of Treg and Th17 cells balance in the development of acute and chronic hepatitis B virus infection. BMC Gastroenterol. 2012;12:43.
47. Haeryfar SM, DiPaolo RJ, Tscharke DC, Bennink JR, Yewdell JW. Regulatory T cells suppress CD8+ T cell responses induced by direct priming and cross-priming and moderate immunodominance disparities. J Immunol. 2005;174:3344–51.
48. Oliphant S, Lines JL, Hollifield ML, Garvy BA. Regulatory T cells are critical for clearing influenza a virus in neonatal mice. Viral Immunol. 2015;28:580–9.
49. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, et al. A distinct function of regulatory T cells in tissue protection. Cell. 2015;162:1078–89.
50. Harb H, Benamar M, Lai PS, Contini P, Griffith JW, Crestani E, et al. Notch4 signaling limits regulatory T-cell-mediated tissue repair and promotes severe lung inflammation in viral infections. Immunity. 2021;54:1186–1199.e7.
51. Suvas S, Azkur AK, Kim BS, Kumaraguru U, Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol. 2004;172:4123–32.
52. Freyschmidt EJ, Mathias CB, Diaz N, MacArthur DH, Laouar A, et al. Skin inflammation arising from cutaneous regulatory T cell deficiency leads to impaired viral immune responses. J Immunol. 2010;185:1295–302.
53. Fulton RB, Meyerholz DK, Varga SM. Foxp3+ CD4 regulatory T cells limit pulmonary immunopathology by modulating the CD8 T cell response during respiratory syncytial virus infection. J Immunol. 2010;185:2382–92.
54. Lanteri MC, O'Brien KM, Purtha WE, Cameron MJ, Lund JM, Owen RE, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. J Clin Invest. 2009;119:3266–77.
55. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med. 2003;198:889–901.
56. Dietze KK, Zelinskyy G, Gibbert K, Schimmer S, Francois S, Myers L, et al. Transient depletion of regulatory T cells in transgenic mice reactivates virus-specific CD8+ T cells and reduces chronic retroviral set points. Proc Natl Acad Sci U S A. 2011;108:2420–5.
57. Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. J Exp Med. 2010;207:1409–20.
58. Suffia IJ, Reckling SK, Piccirillo CA, Goldszmid RS, Belkaid Y. Infected site-restricted Foxp3+ natural regulatory T cells are specific for microbial antigens. J Exp Med. 2006;203:777–88.
59. Zelinskyy G, Dietze KK, Husecken YP, Schimmer S, Nair S, et al. The regulatory T-cell response during acute retroviral infection is locally defined and controls the magnitude and duration of the virus-specific cytotoxic T-cell response. Blood. 2009;114:3199–207.
60. Lund JM, Hsing L, Pham TT, Rudensky AY. Coordination of early protective immunity to viral infection by regulatory T cells. Science. 2008;320:1220–4.

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