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Mini-Review

Regulation of adaptive immune responses to self-antigens in cancer and autoimmunity

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ABSTRACT

Our immune systems are the product of an orchestrated developmental pathway from pluripotent bone marrow cells to selective events in the thymus and periphery. Collectively, innate immunity combined with highly specific adaptive B and T cell responses both protect the host from infection and, at times from tumors, and may also lead to autoimmune pathology. Survival of the host relies on both an efficient and vigorous response from lymphocytes but also upon the attenuation of these responses by regulatory B and T cell subsets. Herein we summarize the recent findings in CD4 and CD8-bearing regulatory T cell functions as well as regulation by IL-10 producing B cell subsets and NKT cells in the context of both tumor immunity and in autoimmunity. Regulatory T cells (Tregs) and/or regulatory B cells (Bregs) control the homeostatic balance of immunity versus chronic inflammation. We will identify factors that may disrupt this balance, both from tumor microenvironments and in local autoimmune milieu. While it is clear that CD4+ Tregs represent a unique developmental lineage with specific markers, discreet markers of Bregs do not yet exist and CD8+ Tregs phenotype and function remains unresolved. Moreover, both B and T cell depletion therapies in autoimmune disease and in immune therapy against solid tumors illustrate a complicated balance of effector and regulatory subsets in maintaining homeostasis. As this field rapidly develops, better markers of regulatory cell phenotypes and manipulation of regulatory lymphocytes will undoubtedly alter therapeutic intervention in cancer and autoimmunity.

KEYWORDS: T regulatory cells (Tregs), B regulatory cells (Bregs), NKT regulatory cells (NKTregs), cancer, autoimmunity, self-antigens, vaccine

INTRODUCTION

Our immune system evolved to simply and effectively clear foreign pathogens in protecting the host from infection. Other immune functions of significance, both beneficial and detrimental to the host, include the surveillance and clearance of tumors, the induction of autoimmunity, and the establishment of immunologic memory. All of these functions share many common interactive pathways responsible for early triggers of the host immune response as well as dampening immune responses once an infection or tumor is cleared. As illustrated in many studies, aberrations in these on-and-off pathways can lead to a variety of both organ specific and systemic autoimmune syndromes, such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE). Lymphocytes that regulate adaptive immune responses have drawn increased attention in the context of therapeutic targeting to induce immune responses to tumors or prevent the development of autoimmunity.

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CD4 Tregs in tumor immunity

Over the past decade the active role of CD4+ Treg cells in tumor immunity has been well established as attested to by numerous detailed reviews on the subject. Therefore, we will only briefly discuss this subset of Treg cells in the context of tumor immunity as it relates to therapeutic modulation. Suppressor thymic-derived T lymphocytes were first described by Gershon and colleagues more than 40 years ago [1, 2]. This group also identified the first T suppressor cell subset among the T-helper cell population (now known to be CD4+) [3]. Some 20 years later this somewhat elusive suppressor T cell population was finally defined. Two main phenotypes of CD4+ Treg cells have been described, those that express increased levels of $CD25^{hi}$ and the Foxp3 transcription factor (classic CD4+ Treg cells) and those that do not, but whose defining characteristic is the production of the immune suppressive cytokine IL-10 (CD4+ Tr1 cells) [4].

The importance of CD4+ Treg cells in suppression of tumor immunity is widely accepted. The major phenotype of these Treg cells includes expression of CD4+ $CD25^{hi}$ Foxp3+ and secretion of the anti-inflammatory cytokines IL-10 and TGF-β1 [5]. Injection of anti-CD25 mAb (PC-61) to improve tumor immunity through transient blocking of CD4+ Treg cells has been recently reported in multiple murine models of cancer, which includes our own work [6-8]. These studies illustrate that vaccination in combination with Treg cell modulation may be critically important for enhancing vaccines that target over expressed tumor self-antigens (reviewed in [9]). Immune therapies that are applied in the presence of tumors to shrink the existing tumor mass may also be affected by mechanisms of suppression other than CD4+ Treg cells. Efforts to better understand tolerance mechanisms in the tumor microenvironment have identified myeloid-derived suppressor cells (MDSCS) as key players in the suppression of therapeutic immunologic strategies (reviewed in [10]). Given the overall failures of therapeutic vaccines and rapid advances in early detection at the point of minimal or no tumor burden, administration of preventive vaccines could be the next immunologic advance in cancer treatment or prevention. The immune suppression described in the presence of a tumor mass may be different than immune suppression elicited by vaccination, where there is no tumor mass or tumor microenvironment. Whether vaccines are applied to treat or prevent cancer, it is clear at least that CD4+ Treg cells are key controllers of immunologic tolerance to over expressed tumor-self proteins (reviewed in [11]).

CD4 Tregs in autoimmunity

Effector CD4+ T cells are central to the development of adaptive autoimmune responses, including multiple sclerosis (MS), myasthenia gravis (MG), rheumatoid arthritis (RA), and SLE. Conversely, CD4+ Tregs are also required to control effector CD4+ autoimmunity as well as innate, TLR7 and TLR9 immune responses that are important in the initiation of SLE. As a generally observed theme in human autoimmune syndromes, overall peripheral Treg numbers are most frequently 'normal' however, *in vitro* measured suppressor activity toward effector CD4+ T cells is typically defective. Illustrated in several mouse models, depletion of $CD25^{hi}$ CD4+ and/or Foxp3+ T cells leads to florid autoimmune pathology in many tissues, including diabetes, oophoritis, arthritis, lupus-like autoimmunity, and gastritis [12-15]. Similarly, human genetic mutations in Foxp3 (IPEX; immune dysregulation, polyendocrinopathy, enteropathy, X-linked) causing functional defects in Foxp3 nTreg populations lead to multi-organ autoimmunity, including autoimmune enteropathy, Type 1 diabetes, dermatitis, thyroiditis, allergy and B cell autoimmunity (anti-insulin, anti-thyroglobulin, anti-platelet, anti-smooth muscle autoantibodies) (reviewed in [16]). The latter observation implicates a role for CD4+ Foxp3+ Tregs in ablating B cell autoimmunity.

MS is a tissue specific autoimmune disease characterized by the activation and infiltration of Th1 and Th17 T cells leading to progressive demyelination of the central nervous system (CNS). Clinical expression of disease is cyclic, noted by spontaneous remission followed by relapses coincident with impaired Treg responses, found both in human disease and in murine models. In the latter model, depletion of $CD25^{hi}$

Tregs amplifies severity of clinical MS while the adoptive transfer of $CD25^{hi}$ Tregs ameliorates disease (reviewed in [17] and [18]). Studies of specific Treg populations from the periphery are problematic in that they rarely reflect cellular interactions at sites of pathology. Indeed, early studies of peripheral $CD25^{hi}$ Treg numbers were found to be no different between MS patients and control subjects, though elevated numbers were found in patient spinal fluids [19-21]. Thus, like in real estate, location is everything in the biology of Tregs. Relapsing remitting MS is marked by a loss of suppressive function by Tregs with lower Foxp3 activity and CTLA-4 expression, not unlike the phenotype of IPEX Tregs [22, 23]. Further evidence suggests that Tregs may differentiate into Th1-like pro-inflammatory phenotypes that secrete IFN-γ in the course of immune dysregulation in MS and also in T1D [24, 25]. Finally, the plasticity of Treg differentiation to Th17-like phenotypes, driven in response to an inflammatory cytokine milieu of IL-17 and IL-6 may also contribute to pathology in MS as well as in SLE, systemic sclerosis, and psoriasis [26, 27].

These studies show that manipulation of the cytokine environment is a viable therapeutic strategy in altering Treg phenotypes and the course of disease in autoimmune syndromes. In RA, CD4+CD25+ Tregs fail to inhibit production of the pro-inflammatory cytokines, TNF-α and IFN-γ from effector CD4+ T cells and this may explain the efficacy of TNF blocking antibodies in the treatment of RA [28]. The role of TNF-α in Treg maintenance and function is controversial, with some studies showing TNF-α downmodulation of Tregs *in vitro*, while others showing the opposite. As with MS, peripheral numbers of Foxp3+ Tregs do not differ in RA and controls, though higher numbers are found in the synovial fluids. Also similar to other autoimmune syndromes, lower Treg CTLA-4 expression in RA marks their reduced suppressor functions.

Considerable research attention has been directed at understanding Treg biology in SLE. As a systemic disease, understanding cellular interactions in microenvironments has proven more difficult, unlike tissue specific syndromes like MS, T1D, or RA where sites of pathology can be specifically studied. As with RA, effector CD4+ T cells are often resistant to Treg inhibition [29]. However, the fact that CD4+ effector T cells are hyper-reactive to TCR mediated stimulation, relative to controls, may offset the ability of Tregs to inhibit. Virtually all murine models of SLE are inhibited by the adoptive transfer of Tregs with reduced autoantibody production, as well as reduced nephritis and cutaneous disease. Similar to other murine models of autoimmunity, MS and T1D, depletion of $CD25^{hi}$ CD4+ T cells causes acceleration of SLE pathology and increased titers of anti-DNA and anti-snRNP autoantibodies, characteristic of disease. In murine SLE models, the NZBxNZW F1 mouse, a progressive imbalance (decrease) of dividing Treg versus CD4+ effector T cell populations in the periphery, spleen, and lymph nodes occurs coincident with age and disease progression. This decline in Treg populations and function are presumed due to a loss of IL-2, required for Treg development and maintenance, in autoimmune prone mice. Interestingly, no such alteration of thymic Foxp3+ occurs over the development of disease. To date, the mechanisms are not fully understood as to how Treg populations become altered over time in selective tissues in murine SLE. While data are conflicting, some studies illustrate the lack of Treg functionality in the progression of murine SLE [30].

Treg biology examined in human SLE is similarly conflicting and/or contradictory which may be due to differences in phenotyping of human Treg populations, since Foxp3 and CD25 can be transiently expressed on activated effector T cell populations in the periphery. Human SLE is complicated by factors known to alter the disease, including genetics, gender, environmental and stochastic variables. Depending on specific markers of human Tregs defined earlier, pure numbers of peripheral Tregs have been found to be relatively normal in some studies, though conflicting data exists in the literature. IL-2 production normally critical for Foxp3 expression and Treg development and maintenance is reduced in human SLE which may account for reduced peripheral $CD25^{hi}$ populations. More recent approaches to defining Treg populations in humans, including methylation

levels of the TSDR region in CD25^{hi} Foxp3+ T cells have only recently been undertaken (reviewed in [15]). Moreover, human SLE is characterized by increased numbers of CD4+ Foxp3+ T cells, considered negative for CD25 (CD25^{low}) though they do not fully exhibit conventional Treg functionality [31]. In fact, these latter Foxp3+ CD25^{low} cells produce inflammatory cytokines, including IFN-γ, IL-4, and IL-17 and it is not clear whether they represent aberrant Treg populations or a novel subset that specifically drives SLE autoimmunity. Human SLE is marked by elevated IL-6 from dendritic cells which inhibits Treg function alone, and together with TGF-β1, drives Th17 inflammatory cells. In summary, microenvironments high in inflammatory cytokines (IL-6, IL-17, IFN-γ) combined with chronic activation of B and T lymphocytes likely overwhelm the ability of IL-2 starved Tregs to control. TGF-β1, often decreased in human SLE, is required for the development of CD4+ Tregs in the periphery, in addition to its role in down regulation of effector CD4+ T cells and inhibition of autoantibody production by B cells.

CD8 Tregs

CD8+ Treg cells have been reported in association with autoimmunity, infectious disease and cancer (reviewed in [32-34]). The phenotypes of CD8+ Treg cells include CD25^{hi}, Foxp3+ [35], CD122^{hi}, IL-10+ [36, 37], non-classical MHC-Qa-1-restricted [38], and those with no apparent antigen specificity [39]. Recent studies suggest that CD8+ CD25^{hi} (IL-2Rα chain) Foxp3+ Treg cells, like CD4+ CD25^{hi} Foxp3+ Treg cells, suppress immunity via cytokines other than IL-10 [40]. Conversely, CD8+ CD122^{hi} (IL-2Rβ chain) Treg cells produce IL-10 to suppress CD8+ T cell effector function [41]. This mechanism of suppression has been demonstrated by Ab-mediated depletion of CD122^{hi} cells *in vivo* [37, 42], transfer of CD8+ CD122^{hi} cells *in vivo* and with CD122$^{-/-}$ knockout mice (it is worth noting that CD122$^{-/-}$ mice have severe immuno-proliferative disease and die by 3 months of age) [43, 44]. CD8+ CD122^{hi} Tregs are reported to mediate their regulatory function via cell-to-cell contact involving classical MHC class I-restriction (reviewed in [36]). The current discrepancies suggest that CD8+ Treg cells come in multiple varieties which may include distinct lineages or sub-types within a suppressor population.

CD8+ CD122^{hi} Treg cells have been reported to spontaneously arise in experimental autoimmune encephalomyelitis (EAE) mice as well as in humans with MS (reviewed in [45]). *In vivo* depletion of CD8+ CD122^{hi} Tregs in EAE mice exacerbates disease, but transfer of this type of CD8+ Treg cells mitigates EAE [42]. In MS patients a subtype of CD8+ Tregs were shown to kill myelin-reactive CD4+ T cells via an HLA-E (non-classical MHC-I) self-peptide along with CD94/NKG2A interaction [46]. Similar HLA-E-restricted CD8+ Treg cells have been described in association with immune responses against tumors in cancer patients (reviewed in [47]). The murine counter-part to human HLA-E-restricted, CD94/NKG2A interacting CD8+ Treg cells seem to be the well characterized CD8+ MHC class Ib Qa-1-restricted (murine equivalent of HLA-E in humans) Tregs (reviewed in [38]). In the case of autoimmunity these two CD8+ Treg cell populations seem to be dominant but use distinct mechanisms of suppression. CD8+ CD122hi Tregs secrete IL-10 to suppress immunity whereas non-classical MHC class Ib-restricted CD8+ Treg cells kill effector T cells via perforin/granzyme secretion (reviewed in [38]).

Unlike what has been reported for CD8+ Treg cells in autoimmune disease, there is a paucity of reports and discord on the phenotype and mechanism of action of CD8+ Treg cells associated with cancer immunity. We have been studying vaccine-induced immunity targeting the novel over expressed tumor self-antigen, Tumor Protein (D52). D52 represents a tumor antigen that exhibits oncogenic properties, and is shared by many cancers (reviewed in [48]). D52 is a unique, over expressed tumor self-protein actively involved in transformation, leading to increased proliferation and metastasis, whose over-expression has been demonstrated in numerous human cancers [49, 50]. The murine orthologue of D52 (mD52) mirrors normal tissue expression patterns of human D52, and shares 86% identity at the amino acid level [51]. We were the first to demonstrate that mD52 vaccination induces protection against tumor challenge without

autoimmunity [6, 52, 53]. We have tested 12 different D52 vaccines in 3 mouse strains against 6 different tumors, with tumor protection ranging from 30% to >70%. Augmented tumor protection required CD4+ $CD25^{hi}$ Treg cell depletion. In all cases we observed vaccine induction of class I MHC-restricted CD8+ IL-10+ T cells that may be a unique subset of suppressor CD8+ T cells involved in tolerance maintenance against broadly expressed tumor-self antigens [6, 52, 53]. The few reports on CD8+ Tregs in tumor immunity describe the expression of $CD122^{hi}$ and/or secretion of TGF- β1 as being common [36, 37]. Our extended analyses of the T cell response to D52 vaccination indicates that the unique CD8+ Tregs cells that are elicited do not produce TGF-β1, are not $CD122^{hi}$, not Foxp3+, and not $CD25^{hi}$ [54]. In fact the only cytokine produced in significant amounts is IL-10 [6, 53, 54]. We believe that this unique population of CD8+ IL-10+ T cells are suppressor rather than effector T cells that actively inhibit immunity to over expressed tumor self-proteins and may be the counterpart to the well described CD4+ Tr1 cells, with the single difference that these unique CD8+ Treg cells also produce perforin and granzyme b (Figure 1). Is it possible that the unique CD8+ Treg cells we observed are Tc10 cells described in association with preventing peripheral tissue damage at the site of active T cell responses against viral pathogens [55]? Tc10 cells are believed to be a transient, reversible phenotype, not a divergent effector lineage [56], supporting the notion that CD8+ T cells that produce IL-10 may be initially IFN-γ producing effector cells that convert to IL-10 production to re-establish homeostasis, as opposed to distinct CD8+ suppressor cells [57]. Tc10 cells have been shown to produce IFN-γ and IL-10 at the same time and may function to protect against brain damage in MS patients [58]. Distinct CD8+ $CD122^{hi}$ IL-10+ Treg cells that produce perforin and granzyme-b, but not IFN-γ, may function to kill autoreactive T cells in models of autoimmune inflammatory bowel disease [44]. The CD8+ IL-10+ Treg cells we described are not likely to be Tc10 cells but are instead a unique subset of CD8+ Treg cells that produce IL-10 and express high levels of perforin and granzyme-b, and lack expression of Foxp3 (Figure 1) [54]. In addition, $CD122^{hi}$ expression is not likely a marker for these CD8+ IL-10+ T cells, since *in vivo* depletion of $CD122^{hi}$ T cells did not augment tumor immunity, but instead exacerbated tumor growth [54]. Finally, the CD8+ IL-10+ T cells elicited in our tumor vaccine model are restricted by classical MHC-I [9, 52, 53, 54] and therefore distinct from non-classical MHC-Ib, Qa-1-restricted CD8+ Tregs [38].

Bregs

B lymphocytes are now appreciated for much more than their ability to secrete Ig. Among those other duties of B cells are antigen presenting cell functions, the transfer of Ig-retained antigens (or autoantigens) to dendritic cells and macrophages, and to function as generalized suppressor cells [59-62]. The existence of B cells that suppress T cell responses was first reported forty years ago [63]. Since then the regulatory B cell subset (Bregs) has been shown to suppress immunity against self-proteins primarily by the production of IL-10 (reviewed in [62, 64]). Breg cells have been described in association with autoimmune disease (reviewed in [65]) and cancer (reviewed in [66]). Indeed, the efficacy of B and T cell depletion therapies in the treatment of autoimmunity illustrate the complicated balance of effector and regulatory subsets in disease. For example, B cell depletion therapies, rituximab and belumimab, generally reduce the severity of rheumatoid arthritis and SLE in spite of the fact that Bregs are likely depleted in the process. Conversely, B cell depletion therapy has been observed to amplify disease in selected cases of human MS and ulcerative colitis, supporting a role for active regulation of autoimmunity by Bregs.

There has yet to be described a Breg specific marker or even a set of specific markers that can be used to identify Bregs in a manner similar to CD4+ $CD25^{hi}$ Foxp3+ Tregs (reviewed in [67]). There are several surface markers that have been described on human and mouse Breg cells most of which are also expressed on other B cell subsets. The precursor lineage of Breg subsets is not yet understood. Established Breg markers include but

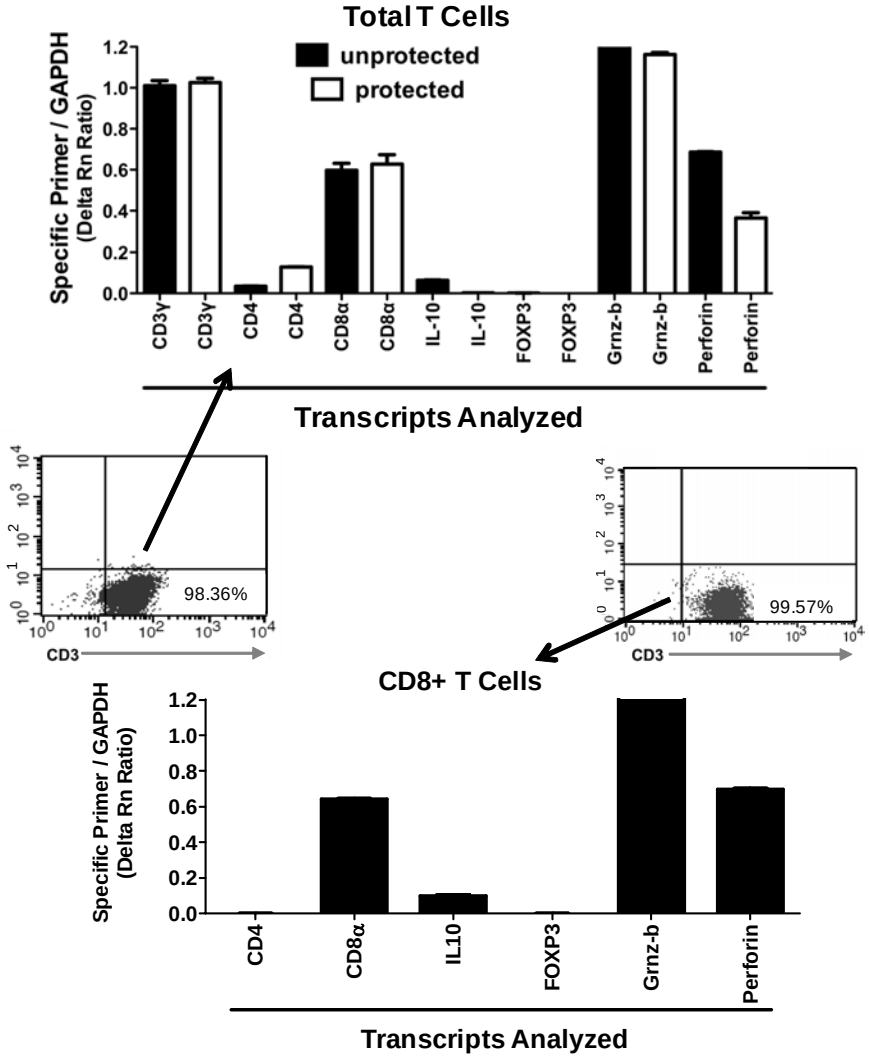

Figure 1. Relative expression of multiple immunologic genes in T cells from D52 immunized mice. The X axis depicts expression of the genes shown relative to GAPDH for a given T cell population determined by real-time RT-PCR at 27 cycles. Upper panel depicts total T cells, Lower panel depicts CD8+ T cells. Data are from representative mice randomly selected from N = 10 mice that were immunized with mD52 DNA and challenged with TRAMP-C2 tumor cells. □ = T cells from immunized mice that were protected from tumor challenge. ■ = T cells from immunized mice that were not protected from tumor challenge. Values = mean +/- SEM for triplicates. Shown are representative data from two separate experiments. Histograms depict representative flow cytometry data demonstrating that the cells analyzed were greater than 98% T cells.

are not limited to CD1d, CD5, CD19, CD21, IgD, and IgM. CD1d is one marker of marginal zone (MZ) B cells, suggesting that they may be precursors of Bregs. Indeed, MZ B cells provide protection from colitis [68]. Some of these surface markers are unique to humans and others are shared between human and mouse Bregs [69, 70]. Thus, IL-10 production remains to be a key definitive marker for both mouse and human Breg cells [67]. IL-10 secretion with costimulation by Bregs or other lymphocytes are signals for CD4+ Treg differentiation, for Foxp3 expression or to inhibit Th17 and Th1 development. Thus, IL-10 producing Bregs may be an important co-factor of Treg development as well as exert indirect suppression of autoimmune states dependent on Th17 and/or Th1 cells (such as SLE and colitis). B cell depletion therapy, particularly rituximab,

has not provided clinical endpoints in ameliorating human SLE. It is tempting to speculate that the absence of Bregs, known to produce higher than normal amounts of IL-10, may no longer control Th17 development in disease.

Regulation of autoimmunity by IL-10 producing B cells has been identified in a variety of models of autoimmunity including EAE, ulcerative colitis, and collagen induced arthritis [71-74]. Notable from several studies of relapsing/remitting EAE, either total B cell depletion or the elimination of IL-10 in hosts prevents remission of disease, while reconstitution of EAE mice with Bregs triggers remission [72, 75]. The stimuli for Breg production of IL-10 includes: B cell receptor, CD40, as well as MyD88-mediated TLR [76, 77]. Chronic expression of EAE is triggered in the specific absence of Myd88 in B cells. Similarly, B cell depletion or the presence of IL-10 deficient B cells triggers chronic expression of EAE.

Bregs may not yet be fully appreciated in their role in maintaining immune tolerance and controlling human autoimmune disease. In human relapsing remitting MS, IL-10 production by activated B cells is significantly decreased relative to control individuals, though some therapies, including IFN-γ, appear to restore B cell cytokine release [74]. B cells in MS and in human SLE fail to normally respond to CD40 stimulation, a critical signal to Breg development. CD154 (CD40L) is overexpressed on SLE T cells which may lead to over stimulation of Breg populations and eventual clonal exhaustion. Breg population dynamics is further complicated by the inflammatory microenvironments found in SLE and MS. Clearly, strong IL-17 (Th17), IL-6, TNF and type 1 IFN responses characteristic of SLE, may simply overwhelm Breg counter regulation. As noted elsewhere, Breg functions require a combination of triggers from BCR, CD40, TLRs and cytokines such as IFN-γ and IL-21. Finally, IL-10 producing Bregs are amplified through other pathways, including calcium stromal interaction molecules (STIM1 and STIM2) and BLNK (SLP65) (reviewed in [67, 78]). It is clear that a better understanding of the phenotypic markers, signaling pathways, signature transcription factors and locations (marginal zone versus follicular or peripheral sites) that delineate Breg populations is required to fully determine their role in the maintenance of immune tolerance.

NKTregs

NKT cells are T cells that, unlike conventional T cells that recognize peptide antigen, recognize lipid antigens in the context of the non-classical MHC class 1b molecule CD1d. NKT cells are sub-classified as either Type I or Type II depending on defined TCR usage and specific lipid antigen recognition (reviewed in [79]). Invariant NKT (iNKT) cells, like Type I NKT cells, have been shown to express CD8 in humans and mice [80], whereas both Type I and Type II NKT cells express CD4. A role for NKT cells in tumor immunity has been described as cross-regulation, where NKT Type I cells generally enhance cellular immunity against tumors and NKT Type II cells suppress tumor immunity through a detailed three-way interaction that involves classic CD4+ $CD25^{hi}$ Foxp3+ Treg cells (reviewed in [79]). In this scenario NKT Type II cells would be considered a type of NKTreg cell and NKT Type I cells an effector cell population. Conventional Type I NKT cells are commonly defined by TCR Vα14 Jα18 expression in mice and Vα24 Jα18 in humans, as well as α-GalCer antigen specificity [79, 81]. A recent report describes a distinct subset of NKT Type I cells that produce IL-10 (NKT10 cells) and are immune suppressive in a mouse model of EAE [81]. Similar to Bregs and subsets of Tregs, IL-10 seems to be a key suppressive factor associated with NKT10 cells.

CONCLUSION

Here in we highlighted the current understanding of the best characterized lymphocyte populations that have been shown to play roles in the active regulation/suppression of adaptive immune responses to self-antigens, namely Treg cells (CD4+ and CD8+), Breg cells and NKTreg cells. Each of these regulatory lymphocyte populations has individually been the focus of many detailed reviews most notably CD4+ Treg cells. Thus, our primary intent in writing this mini-review was to bring together, as a single source, collective

information on these regulatory lymphocytes and their roles in autoimmune disease and cancer. A picture, of how regulatory lymphocytes function and how their phenotypes differ from their effector cell counter part, is becoming clearer. Much has been done to provide a deeper understanding of CD4+ Treg cells. Bregs and NKTregs with defined phenotypes and functions are still at the beginning stages of discovery. Definitive markers for Breg cell populations have yet to be discovered, and the exact phenotypic relationship of NKTregs with NKT10 cells and iNTK cells remains unclear. Unlike CD4+ Tregs, which are well defined phenotypically and functionally at least apart from other regulatory lymphocytes, CD8+ Tregs remain the least characterized regulatory lymphocyte particularly in the context of tumor immunity to self-antigens. There appear to be some CD8+ Tregs that are $CD122^{hi}$, or that are $CD25^{hi}$ and Foxp3+, and some that are uniquely restricted to the species related, non-classical MHC molecules HLA-E (humans) and Qa-1 (in mice). There is also some early evidence that a distinct CD8+ Treg cell population exists that shares none of the markers currently described for other CD8+ Treg cells and may play a unique role in maintaining tolerance to widely expressed self-antigens that are over-expressed in tumor cells. All of these CD8+ Tregs appear to be distinct from each other in phenotype, in function and role. Interestingly, what has emerged as a common functional link among all regulatory lymphocytes whether T, B or NKT is the expression and use of the immune suppressive cytokine IL-10. There remains a need to understand how these distinct groups of regulatory lymphocytes that share the common goal of maintaining tolerance to self-antigens work in concert during the complex firestorm of an immune response; perhaps IL-10 is a key to their interactions. When all the regulatory lymphocyte lineages are as individually well characterized and defined as the CD4+ Treg cell populations, then the challenging and important task of studying the intricate control of self-tolerance mediated by the simultaneous contributions of each of the distinct regulatory lymphocyte populations can begin.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

ABBREVIATIONS

Treg, T regulatory cell; Breg, B regulatory cell; NKTreg, NKT regulatory cell; TPD52, tumor protein D52; EAE, experimental autoimmune encephalomyelitis; SLE, systemic lupus erythematosus; MS, multiple sclerosis; MG, myasthenia gravis; RA, rheumatoid arthritis; T1D, Type 1 diabetes; MDSC, myeloid-derived suppressor cells.

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