Cellular & Molecular Immunology (2010), 1–6

www.nature.com/cmi

REVIEW

Multiple checkpoints keep follicular helper T cells under control to prevent autoimmunity

Di Yu[1] and Carola G Vinuesa[2]

Follicular helper T (Tfh) cells select mutated B cells in germinal centres, which can then differentiate into long-lived high affinity memory B cells and plasma cells. Tfh cells are regulated by a unique molecular programme orchestrated by the transcriptional repressor Bcl6. This transcription factor turns down expression of multiple genes, including transcriptional regulators of other T helper lineages and a vast amount of microRNAs. This enables Tfh cells to express a suite of chemokine receptors, stimulatory ligands and cytokines that enable migration into B-cell follicles, and provision of effective help to B cells. Not surprisingly, dysregulation of this powerful helper subset can lead to a range of autoantibody-mediated diseases; indeed, aberrant accumulation of Tfh cells has been linked with systemic lupus erythematosus, Sjogren's disease and autoimmune arthritis. Here we dissect multiple checkpoints that operate throughout Tfh cell development and maturation to maintain immunological tolerance while mounting robust and long-lasting antibody responses.
Cellular & Molecular Immunology advance online publication, 5 April 2010; doi:10.1038/cmi.2010.18

Keywords: autoimmunity; germinal centre; Tfh

INTRODUCTION

The enormously diversified repertoire of T-cell receptors (TCRs) capable of recognising innumerable combinations of peptide–major histocompatibility complex molecules, enable the adaptive immune system to fight any invading pathogen. The trade-off of the random process of receptor diversification is the unavoidable recognition of self-antigens with potentially dangerous affinity. The adaptive immune system faces the constant challenge of maintaining tolerance to self-components of the body while mounting robust responses to foreign pathogens. $CD4^+$ helper T (Th) cells orchestrate mammalian adaptive immunity and are subjected to several tolerance checkpoints from their earlier developmental stages in the thymus, through to their maturation in the periphery and differentiation into effector and memory subsets. The checkpoints that are common to all $CD4^+$ helper subsets include: (i) clonal deletion of self-reactive T cells in the thymus; (ii) biochemical tuning that limits activation downstream of chronic TCR signalling from self-reactive receptors; (iii) limiting immunogenic costimuli; (iv) control of the lifespan of the effector populations[1] and (v) suppression of immune responses by regulatory T (Treg) cells.[2] Defects in any of these tolerance mechanisms can lead to a wide range of systemic and organ-specific autoimmune diseases. Further tolerance mechanisms exist to control the accumulation and function of individual $CD4^+$ helper subsets. Those that pertain to follicular helper T (Tfh) cells have emerged as being critical to prevent autoantibody-driven autoimmune diseases and are the subject of this review.

Tfh cells were originally identified as a subset of Th cells found to reside in close proximity to follicular dendritic cells within germinal centres.[3] Soon after, their phenotype was described to differ from that of Th1 and Th2 cells.[4] It took more than 20 years to place Tfh cells in a subset separate from other helper subsets: the recent identification of Bcl6 as the transcriptional regulator of Tfh cells has firmly established these cells as an independent lineage. This was supported by the demonstration that Th1, Th2 and Th17 cells develop normally in the absence of Bcl6 but Tfh cells were completely dependent on this transcription factor.[5–7] The unique ability of Tfh cells to select mutated high affinity B cells destined to live for decades in an individual places them at a critical vulnerable spot for immunological tolerance.

The process of somatic hypermutation typically occurs in germinal centres—although it can also occur at extrafollicular sites in autoimmune-prone mice—and targets immunoglobulin (Ig) variable region genes of rapidly dividing germinal centre B cells (centroblasts).[8] This can lead to an increase in the affinity of the B-cell receptor for the immunising antigen, but there is abundant evidence that this stochastic process can also generate self-reactive specificities.[8] Furthermore, once self-reactive B cells have been vaguely selected in germinal centres, their differentiated offspring can live and produce antibodies unchecked, subject to virtually no further control. It has been long known that most anti-double stranded DNA antibodies detected in humans and in animal models of systemic lupus erythematosus (SLE) are high-affinity IgG antibodies, which suggests that they may have been generated in germinal centre reactions.[9] Thus, a tightly controlled process of germinal centre B-cell selection by antigen-specific Tfh cells is normally in place to ensure positive selection of those cells

[1]Department of Immunology and Inflammation, Garvan Institute of Medical Research, Sydney, NSW, Australia and [2]Department of Immunology and Genetics, John Curtin School of Medical Research, Canberra, ACT, Australia
Correspondence: Dr D Yu, Department of Immunology and Inflammation, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia.
E-mail: d.yu@garvan.org.au
Received 3 February 2010; accepted 28 February 2010

with the highest affinity towards foreign antigens while preventing selection of cells that have become self-reactive. The randomness of the mutation process, together with the longevity of post-germinal centre memory B cells and plasma cells, and the abundance of exposed nuclear antigens on the surface of numerous germinal centre cells undergoing apoptosis, rank germinal reactions the highest in the risk of triggering and maintaining autoantibody-driven autoimmunity.[8]

While Tfh cells are to a large extent responsible for maintaining tolerance in germinal centers, it is important to note this is not the only mechanism operating at this stage of immune responses. Mutated germinal centre B cells that fail to receive prosurvival signals are programmed to die by apoptosis, providing a mechanism that prevents the production of high-affinity, long-lived self-reactive B cells.[10] A critical checkpoint that can rapidly eliminate self-reactive germinal centre B cells is the need to establish an immunological synapse with follicular dendritic cells: germinal centre B cells that bind soluble antigen without receiving integrin-mediated signals from follicular dendritic cells die very rapidly—within 4–6 h.[11,12] In mice that lack Dock8, required to accumulate intercellular adhesion molecule-1 in the B-cell immunological synapse, germinal centres are short-lived.[13] This highlights the need for antigen to be presented in the form of immune complexes, which will readily happen in the case of foreign antigens, but not self-antigens. Germinal centre B cells then need to be selected by Tfh cells.

What are the unique features of Tfh cells that ensure that not only are they completely devoid of self-reactive specificities, but also can ruthlessly eliminate all germinal centre B cells that have acquired crossreactivity against self-antigens during the process of SHM? The answer partly lies in: (i) the Tfh phenotype that facilitates follicular homing and B-cell helper function depends on a sophisticated gene expression programme controlled by Bcl6 that represses multiple target genes including several transcription factors and microRNAs (miRNAs); (ii) strong TCR signalling, which in the presence of normal thymic selection is likely to occur only in response to foreign antigens, favours Tfh differentiation; (iii) Tfh development is dependent on the ability to form highly stable cognate T–B cell interactions; (iv) Tfh cells turn down production of potentially proinflammatory cytokines such as interferon-γ (IFN-γ) and interleukin (IL)-17, and rely on the actions of IL-21 and IL-4 that cooperate with CD40 ligand (CD40L) to maintain germinal centres; (v) Tfh cells, like other T-helper subsets, are also subject to Treg cell control, and (vi) Tfh cells and germinal centre B cells turn on a proapoptotic programme that limits their survival. When any of these features destined to promote self-tolerance and prevent autoimmunity, goes awry, Tfh cells can aberrantly accumulate and/or aberrantly select self-reactive germinal centre B cells. The key regulatory role of Tfh cells in germinal centres has placed this population in the frontline of immunological tolerance. In this review, we will summarise the emerging evidence for participation of Tfh cells in autoimmune diseases and examine the known checkpoints during Tfh development and maturation to prevent autoimmunity. It is important to emphasise that this field is in its infancy, and there is great need to increase our understanding of the molecular mechanisms that enable Tfh cell-mediated selection of high affinity and non-self-reactive germinal centre B cells, and control Tfh differentiation and lifespan.

DEVELOPMENT OF A SELF-REACTIVE TFH CELL COMPARTMENT

In the thymus, TCRs that bind strongly to self-peptide–MHC complexes trigger the death of thymocytes, a process known as negative selection. When this process is defective due to defects in self-antigen presentation, TCR signalling or apoptosis, autoreactive T cells reach the periphery and cause systemic or organ specific autoimmunity.[1]

Failed negative selection of self-reactive T cells resulting in a non-self tolerant Tfh population has been shown to contribute to the autoimmune arthritis of K/BxN mice. These mice develop an aggressive form of arthritis that has many of the clinical, histological and immunological features of rheumatoid arthritis in humans.[14] K/BxN mice were originally generated by crossing KRN TCR-transgenic mice on the C57BL6/J genetic background (K/B) with non-obese diabetic mice. This TCR transgene is specific for a bovine ribonuclease peptide (RNase 42–56) but was subsequently also found to recognise glucose-6-phosphate isomerase (GPI), a ubiquitously expressed self-antigen that is also present on the surface of inflamed joints, presented by the MHC class II molecule I-A^{g7}[14]. KRN $CD4^+$ T cells escape clonal deletion in the thymus and mature T cells appear in the periphery at 3 weeks of age, albeit in reduced numbers.[15] Primed KRN $CD4^+$ T cells provide help to GPI-reactive B cells leading to the production of high titres of anti-GPI antibodies.[14] Expression of the transgenic TCR is an absolute requirement for the development of the arthritic phenotype[14] and adoptive transfer of $CD4^+$ T cells from healthy K/B donors into mice of BxG7 (C57BL/6J × B6.$H2^{g7}$ F1) genetic background was sufficient to initiate germinal centre responses, produce anti-GPI autoantibodies and develop arthritis.[16]

Importantly, this model of autoimmune arthritis is autoantibody-dependent: transfer of K/BxN serum or purified polyclonal GPI antibodies are sufficient to trigger arthritis in *Rag-1*$^{-/-}$ recipient mice.[17] This corroborates that it is the B-cell helper activity of KRN Tfh cells that triggers the autoimmune process. Furthermore, in the adoptive transfer model described above, T cells deficient in CXC-motif chemokine receptor 5 (CXCR5) expression were precluded from entering the follicles, an important step during Tfh-cell differentiation,[18] and essentially lost the capability to induce germinal centre formation, autoantibody production and arthritis development.[16]

ABERRANT TFH ACCUMULATION

Although the signals that drive Tfh formation are not completely understood, it has been shown that strong TCR signalling and inducible costimulator (ICOS)-mediated costimulation favour Tfh differentiation, which also requires a stable signalling lymphocyte activation molecule-associated protein (SLAM-associated protein, SAP)-mediated T–B interaction and is promoted by IL-21.[18,19].

The pathogenic consequences of Tfh cell accumulation in the absence of immunisation have been demonstrated in *sanroque* mice, an *N*-ethyl-N-nitrosourea-induced strain bearing a homozygous point mutation in the *Roquin* gene.[20] *Sanroque* mice develop a systemic autoimmune syndrome with many features typical of SLE including high-affinity anti-double stranded DNA antibodies, focal proliferative glomerulonephritis with deposition of IgG-containing immune complexes, anaemia and autoimmune thrombocytopenia as well as other autoimmune manifestations such as lymphadenopathy, splenomegaly, necrotising hepatitis and plasmacytosis.[20] Spontaneous germinal centre formation is detected in *sanroque* mice as early as 4 weeks after birth, accompanied by massive accumulation of $CD4^+$ T cells in germinal centres. *Sanroque* $CD4^+$ cells express high amounts of CXCR5, programme cell death-1 (PD-1), ICOS and IL-21, characteristic of a Tfh phenotype.[20] *Roquin* was found to act T cell-autonomously to cause Tfh cell accumulation.[21] Adoptively transferred Tfh cells from *sanroque* mice into C57BL/6 mice enhanced germinal centre formation in wild-type recipient mice in the absence of immunisation.

Evidence for a causal role of Tfh dysregulation in the autoimmune phenotype came from the demonstration that *sanroque* mice made genetically deficient in SAP, completely abrogated Tfh cell accumulation and formation of spontaneous germinal centers, prevented lupus-like disease and end-organ damage.[21] Further evidence that *sanroque* lupus is of Tfh/germinal center origin came from studies in which the gene dose of *Bcl6*—the transcriptional regulator of both germinal centre B and Tfh cells—was halved: lupus was significantly ameliorated in *sanroque Bcl6*$^{+/-}$ mice.[21] Taken together, these studies suggest that aberrant positive selection by excessive and dysregulated Tfh cells in *sanroque* mice is a key factor in the development of systemic autoimmunity, suggesting that tight control of Tfh-cell numbers and function is a key checkpoint in the maintenance of immunological tolerance.

We recently identified an increase of otherwise rare circulating Tfh-like (cTfh) CXCR5^{high}ICOS^{high}PD-1^{high} CD4$^+$ T cells in the blood of a subset of SLE and Sjögren's syndrome patients (20–30%). This 'cTfh^{high}' phenotype was stable over time and closely correlated with disease severity. Of note, a comparable cTfh population was seen in the blood of *sanroque* mice, which correlated with the increase in resident Tfh within secondary lymphoid organs.[22] Although cTfh cells from SLE patients do not express high levels of the Tfh transcription factor Bcl6,[22] the similarity in phenotype suggests that they might be either Tfh progenitors capable of terminal differentiation into Tfh cells upon entry into secondary lymphoid tissues, or they derive from Tfh cells that have emigrating from germinal centres into the circulation. Thus, excessive Tfh cell formation may be a common feature of at least a proportion of patients with autoimmune diseases.

Roquin binds to *Icos* mRNA (unpublished observation) and regulates its stability acting in concert with miRNAs.[23] Wild-type *Roquin* represses ICOS post-transcriptionally, but this regulation is impaired by the presence of mutant *Roquin*, leading to ICOS overexpression on *sanroque* CD4$^+$ T cells, which contributes in part to the accumulation of Tfh cells.[23] As the cellular and molecular mechanisms responsible for maintaining tolerance are being deciphered, it is becoming clear that spatiotemporal control of gene expression underpins most successful tolerance mechanisms. miRNAs, as a group of short non-coding RNAs of about 20–22 nucleotides modulating the stability and translational efficiency of target mRNAs, present an important new layer controlling gene expression to prevent different types of polygenic disorders.[24] miRNAs target immune transcripts to fine-tune gene expression and turn on negative feedback loops that maintain the delicate balance between protective versus autoimmune responses.[25,26] Our recent description of Bcl6-mediated repression of over 30 miRNAs, including miR-17–92 shown to repress CXCR5 expression,[7] underscores the role of this layer of regulation in Tfh differentiation. Thus, it is likely that miRNA-regulated checkpoints also operate to prevent autoimmunity of Tfh-germinal centre origin.

FAILED FOLLICULAR EXCLUSION OF UNSUITABLE TH CELLS

Each Th subset has a distinct cytokine production profile: Th1 cells are potent IFN-γ producers, Th2 cells mainly secrete IL-4, IL-13 and IL-15, and Th17 cells produce IL-17F, IL-22 and IL-21.[27] Tfh cells differ from these subsets by their predominant production of two cytokines, IL-21 and IL-4,[28–33] with only low amounts of IFN-γ and IL-17 being produced in the follicles.[7,34] We and others have shown that the transcription repressor Bcl6 directs Tfh differentiation and suppresses the differentiation programme to other Th lineages.[6,7,35] Bcl6 decreases production of IFN-γ, IL-4 and IL-17 probably through the direct repression of the transcription factors T-box 21, GATA-binding protein 3 and retinoic acid receptor-related orphan receptor gamma, thymus-specific isoform.[6,7,35]

Of interest, there is a striking negative correlation between expression of CXCR5 and IL-17 in tonsil CD4$^+$ T cells, which are typically rich in Th1, Th2, Th17 and Tfh cells.[7] This suggests that Th17 cells are excluded from follicular entry and unlikely to provide help to germinal centre B cells. Several lines of experimental evidence support this notion. First, a pathogenic role of IL-17 in dysregulated antibody responses and autoimmunity has been recently reported. SLE patients display increased levels of both IL-17 and B cell-activating factor. Strikingly, IL-17 alone or in combination with B cell-activating factor that enhances human B-cell survival, promotes B-cell proliferation and differentiation into antibody-secreting cells *ex vivo*.[36] This suggests that exclusion of Th17 cells from B cell follicles would be important to prevent them from providing non-cognate survival and differentiation signals to autoreactive germinal centre B cells and lower the threshold for B-cell selection.

A second line of evidence supporting the idea IL-17 may subvert tolerance mechanisms in germinal centres leading to autoimmunity comes from studies of BDX2 mice. BDX2 mice were generated by inbreeding the intercross progeny of C57BL/6J and DBA/2J mice for more than 20 generations.[37] These mice show rising titres of autoantibodies and circulating immune complexes and progressively develop glomerulonephritis and erosive arthritis with age.[38] The pathogenic autoantibodies developed in BDX2 mice are largely dependent on T-cell help and are likely to be the products of germinal centres.[39] The important, yet surprising observation is the accumulation of Th17 cells in BDX2 mice and the fact many of these Th17 cells localise within germinal centres.[40] Introduction of two null alleles of IL-17R ameliorated the spontaneous formation of germinal centres in BDX2 mice, whereas administration of exogenous IL-17 induced germinal centre formation in wild-type mice and exaggerated spontaneous germinal centre formation and autoantibody production in BDX2 mice.[40] It has been suggested that excessive IL-17 upregulates the expression of regulator of G-protein signalling 13 and/or 16 of B cells and causes retention of B cells in germinal centres, allowing them to undergo repeated rounds of somatic hypermutation.[40] This together with a lower selection threshold due to the abundance of T cells and provision of non-cognate survival signals would lead to an environment where self-reactive B cells are aberrantly selected.

EXCESSIVE HELPER SIGNALS TO B CELLS

Tfh-derived helper signals such as CD40L and IL-21 not only sustain germinal centres but also are critical to select mutant B cells and enable them to terminally differentiate into long-lived high affinity memory or plasma cells.[8] The question is: Do varying amounts of these helper signals control germinal centre events and determine the threshold for selection? There is indirect evidence to suggest that this is the case. In humans, overexpression of CD40L[41,42] and IL-21[43] has been reported in SLE patients, suggesting a link between excessive B helper signals and autoimmunity. However, this does not necessarily mean that an excess of these signals exclusively corrupts germinal centre selection, since both CD40L and IL-21 play important roles in extrafollicular antibody responses (see discussion below).

Overexpression of CD40L on T cells enhances thymocyte apoptosis, which causes thymic atrophy and precludes investigating the functional consequences of excessive expression of CD40L in the periphery.[44] To circumvent this problem, CD40L was overexpressed on B cells, and shown to provide autocrine helper signals that resulted in a lupus-like autoimmune disease in mice with spontaneous production

of autoantibodies and development of glomerulonephritis with immune-complex deposition.[45]

IL-21 transgenic mice showed a dramatic increase in the number of post-switch plasma cells and the titres of IgM and IgG1 in sera compared to wild-type littermates.[46] Higher serum levels of IL-21 were also detected in BSXB-*Yaa* mice, a mouse model of SLE with the characteristic lymphadenopathy, splenomegaly, leukocytosis, hypergammaglobulinemia and severe immune complex-mediated glomerulonephritis.[46] Genetic depletion of IL-21R in these mice abrogated the hypergammaglobulinemia, autoantibody production, renal disease and premature morbidity in these mice,[47] suggesting an essential role of IL-21/IL-21R pathway in the pathogenesis of the autoimmune disorder. It appears that both follicular and extrafollicular T cells are important producers of IL-21 in these mice.

As mentioned above, the excessive B helper signals contributing to autoimmunity do not act exclusively in germinal centres, but also perturb extrafollicular B-cell responses. Arguably, the best example is the demonstration of the pathogenic role of IL-21 in the MRL/MpJ-*Fas*^{lpr} (MRL^{lpr}) mouse model of SLE, in which mutated autoreactive plasmablasts grow in extrafollicualr foci. In these mice, IL-21 contributes to the production of pathogenic autoantibodies.[48] An extrafollicular population of ICOS^{high}P-selectin glycoprotein ligand 1^{low} CD4^{+} helper T cells appears to be the primary source of CD40L and IL-21 helper signals supporting extrafollicular development of IgG plasmablasts.[49] The relationship between these extrafollicular helper T cells and Tfh cells is still not fully resolved.

CONTROLLING THE LIFESPAN OF TFH CELLS

Most Tfh cells appear to be short-lived effectors with limited proliferative potential once they enter germinal centres, probably due to decreased expression of antiapoptotic Bcl2 and Bcl$_{xL}$ and increased expression of other proapoptotic Bcl2 family members including Bcl2-antagonist/killer 1, BH3 interacting domain death agonist and Bcl2-associated agonist of cell death. Tfh cells also express high amounts of the tumour-necrosis factor receptor superfamily, member 6 (Fas), and are more sensitive to cell death after TCR stimulation than naïve or memory helper T cells.[50,51] Histological staining reveals that, unlike interfollicular T cells, human tonsillar Tfh cells do not express Bcl2,[52] which is consistent with the reported role for the Tfh-directing transcription factor Bcl6 in repressing Bcl2 expression.[53] Overexpression of Bcl2 in the entire hematopoietic compartment (Bcl2 transgene controlled by *Vav* gene regulatory sequences) instead of selectively in B cells (Bcl2 transgene controlled by an IgH enhancer), results in spontaneous germinal centre formation.[54] The germinal centre hyperplasia was abrogated by CD4^{+} T-cell depletion, suggesting that excessive CD4^{+} T cell longevity was the cause of this phenotype.[54] Although the entire peripheral T-cell compartment was expanded in this mice by about five-fold,[54] it is likely that defective Tfh cell apoptosis is a major contributor to the abnormal formation of germinal centres.

TREG-MEDIATED SUPPRESSION OF TFH CELLS

Treg cells represent a small but *bona fide* population of T cells in germinal centres.[55,56] These Treg cells are capable of suppressing the effects of Tfh cells and may play a role in repressing Tfh function, including IL-17 expression by Tfh cells, thus moderating the provision of help that Tfh cells can provide to B cells within the germinal centres.[55,57]

Of particular interest is the observation that Treg cells, an immunosuppressive population, can differentiate into Tfh cells in the gut of lymphopenic mice.[58] This conversion appears to only take place in Peyer's patches but not in spleen or lymph nodes of the same mice, demonstrating the need of a unique microenvironment to foster Tfh-cell differentiation from Treg cells.[58] However, it is possible that certain conditions or proinflammatory environments may promote Treg to Tfh-cell conversion in secondary and tertiary lymphoid organs.

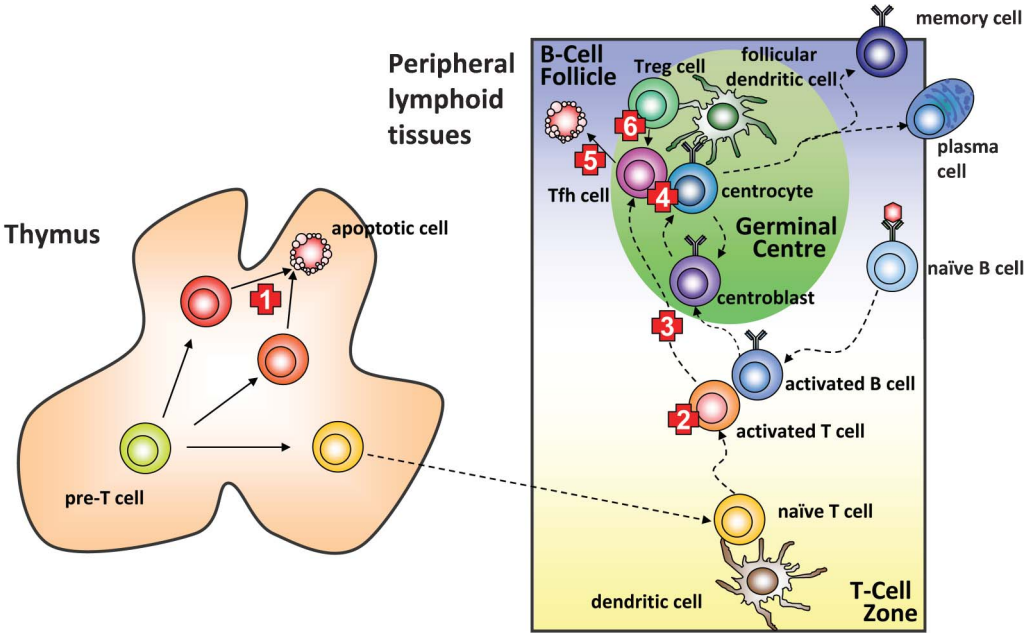

Figure 1 Cellular checkpoints that keep Tfh cells under control to maintain tolerance. Individual checkpoints are shown by numbered red crosses: (1) deletion of immature T cells with high-affinity self-reactive T-cell receptors; (2) limiting extrinsic stimuli and intrinsic signals that promote Tfh differentiation; (3) exclusion of non-Tfh effector populations from the follicles; (4) maintenance of the threshold for germinal centre B-cell selection; (5) short lifespan and proapoptotic nature of Tfh cells; (6) suppression of Tfh cells by Treg cells. Tfh, follicular helper T; Treg, regulatory T.

Ectopic germinal centres are detected in 30–50% of joints from patients with rheumatoid arthritis and the salivary glands from patients with Sjögren's syndrome.[59] Whether Treg to Tfh conversion occurs at those sites or the reasons why it may fail to occur are still not clear and will be an interesting subject of investigation.

CONCLUDING REMARKS

Tfh cells have recently acquired their own identity in the big Th cell family. Growing evidence of their important role in maintaining germinal centre tolerance and demonstration that autoimmunity can arise when they are dysregulated have placed Tfh cells in the limelight of the pathogenesis of autoantibody-driven autoimmune diseases (Figure 1). There are still many unanswered questions regarding Tfh-cell ontogeny, tolerisation, mechanism of action, factors that control their growth and survival, and their relationship with other helper and regulatory subsets. The answers to these questions will hold the key to unravelling how tolerance is maintained during the process of negative and positive selection in germinal centres. Much of the information available to date illuminating the checkpoints that prevent autoimmunity of Tfh/germinal centre origin has been obtained from autoimmune mouse models. The challenge ahead is to translate these findings into information that is valuable to improve the diagnosis and therapy of patients with autoimmune diseases. This will need a much better understanding of human Tfh biology in health and disease.

ACKNOWLEDGEMENTS

We wish to acknowledge constructive discussion with Nina Chevalier, Charles R. Mackay, Jing He and Zhanguo Li. CGV is supported by a Viertel Senior Medical Research Fellowship and NHMRC project and programme grants. DY is supported by a Cancer Institute New South Wales Fellowship, an NHMRC Fellowships and an NHMRC programme grant to CRM.

1 Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 2005; **435**: 590–597.
2 Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat Immunol* 2010; **11**: 7–13.
3 Stein H, Gerdes J, Mason DY. The normal and malignant germinal centre. *Clin Haematol* 1982; **11**: 531–559.
4 Velardi A, Mingari MC, Moretta L, Grossi CE. Functional analysis of cloned germinal center $CD4^+$ cells with natural killer cell-related features. Divergence from typical T helper cells. *J Immunol* 1986; **137**: 2808–2813.
5 Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B *et al*. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 2009; **325**: 1006–1010.
6 Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD *et al*. Bcl6 mediates the development of T follicular helper cells. *Science* 2009; **325**: 1001–1005.
7 Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL *et al*. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* 2009; **31**: 457–468.
8 Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. *Nat Rev Immunol* 2009; **9**: 845–857.
9 Radic MZ, Weigert M. Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu Rev Immunol* 1994; **12**: 487–520.
10 Strasser A, Bouillet P. The control of apoptosis in lymphocyte selection. *Immunol Rev* 2003; **193**: 82–92.
11 Pulendran B, Kannourakis G, Nouri S, Smith KG, Nossal GJ. Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature* 1995; **375**: 331–334.
12 Shokat KM, Goodnow CC. Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature* 1995; **375**: 334–338.
13 Randall KL, Lambe T, Johnson A, Treanor B, Kucharska E, Domaschenz H *et al*. Dock8 mutations cripple B cell immunological synapses, germinal centers and long-lived antibody production. *Nat Immunol* 2009; **10**: 1283–1291.
14 Ditzel HJ. The K/BxN mouse: a model of human inflammatory arthritis. *Trends Mol Med* 2004; **10**: 40–45.
15 Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. *Cell* 1996; **87**: 811–822.
16 Victoratos P, Kollias G. Induction of autoantibody-mediated spontaneous arthritis critically depends on follicular dendritic cells. *Immunity* 2009; **30**: 130–142.
17 Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T *et al*. From systemic T cell self-reactivity to organ-specific autoimmune disease *via* immunoglobulins. *Immunity* 1999; **10**: 451–461.
18 Yu D, Vinuesa CG. What makes Tfh cells special. *Trends Immunol* 2010; in press.
19 Yu D, Batten M, Mackay CR, King C. Lineage specification and heterogeneity of T follicular helper cells. *Curr Opin Immunol* 2009; **21**: 619–625.
20 Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM *et al*. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 2005; **435**: 452–458.
21 Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL *et al*. Follicular helper T cells are required for systemic autoimmunity. *J Exp Med* 2009; **206**: 561–576.
22 Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG *et al*. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum* 2010; **62**: 234–244.
23 Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG *et al*. Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. *Nature* 2007; **450**: 299–303.
24 Tsai LM, Yu D. MicroRNAs in common diseases and potential therapeutic applications. *Clin Exp Pharmacol Physiol* 2010; **37**: 102–107.
25 Vinuesa CG, Rigby RJ, Yu D. Logic and extent of miRNA-mediated control of autoimmune gene expression. *Int Rev Immunol* 2009; **28**: 112–138.
26 Luo X, Tsai LM, Shen N, Yu D. Evidence for microRNA-mediated regulation in rheumatic diseases. *Ann Rheum Dis* 2010; **69**(Suppl 1): i30–i36.
27 Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* 2008; **112**: 1557–1569.
28 Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L *et al*. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 2008; **29**: 138–149.
29 Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity* 2008; **29**: 127–137.
30 Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. *Nat Immunol* 2009; **10**: 375–384.
31 King IL, Mohrs M. IL-4-producing $CD4^+$ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J Exp Med* 2009; **206**: 1001–1007.
32 Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat Immunol* 2009; **10**: 385–393.
33 Zaretsky AG, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J Exp Med* 2009; **206**: 991–999.
34 Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B *et al*. Early commitment of naive human CD4(+) T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12. *Immunol Cell Biol* 2009; **87**: 590–600.
35 Kusam S, Toney LM, Sato H, Dent AL. Inhibition of Th2 differentiation and GATA-3 expression by BCL-6. *J Immunol* 2003; **170**: 2435–2441.
36 Doreau A, Belot A, Bastid J, Riche B, Trescol-Biemont MC, Ranchin B *et al*. Interleukin 17 acts in synergy with B cell-activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus. *Nat Immunol* 2009; **10**: 778–785.
37 Taylor BA, Wnek C, Kotlus BS, Roemer N, MacTaggart T, Phillips SJ. Genotyping new BXD recombinant inbred mouse strains and comparison of BXD and consensus maps. *Mamm Genome* 1999; **10**: 335–348.
38 Hsu HC, Zhou T, Kim H, Barnes S, Yang P, Wu Q *et al*. Production of a novel class of polyreactive pathogenic autoantibodies in BXD2 mice causes glomerulonephritis and arthritis. *Arthritis Rheum* 2006; **54**: 343–355.
39 Hsu HC, Wu Y, Yang P, Wu Q, Job G, Chen J *et al*. Overexpression of activation-induced cytidine deaminase in B cells is associated with production of highly pathogenic autoantibodies. *J Immunol* 2007; **178**: 5357–5365.
40 Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J *et al*. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008; **9**: 166–175.
41 Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J Clin Invest* 1996; **97**: 2063–2073.
42 Koshy M, Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J Clin Invest* 1996; **98**: 826–837.
43 Wong CK, Wong PT, Tam LS, Li EK, Chen DP, Lam CW. Elevated production of B cell chemokine CXCL13 is correlated with systemic lupus erythematosus disease activity. *J Clin Immunol* 2010; **30**: 45–52.
44 Clegg CH, Rulffes JT, Haugen HS, Hoggatt IH, Aruffo A, Durham SK *et al*. Thymus dysfunction and chronic inflammatory disease in gp39 transgenic mice. *Int Immunol* 1997; **9**: 1111–1122.
45 Higuchi T, Aiba Y, Nomura T, Matsuda J, Mochida K, Suzuki M *et al*. Cutting edge: ectopic expression of CD40 ligand on B cells induces lupus-like autoimmune disease. *J Immunol* 2002; **168**: 9–12.
46 Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF *et al*. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J Immunol* 2004; **173**: 5361–5371.

47 Bubier JA, Sproule TJ, Foreman O, Spolski R, Shaffer DJ, Morse HC 3rd *et al*. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice. *Proc Natl Acad Sci USA* 2009; **106**: 1518–1523.
48 Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K. IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R.Fc reduces disease progression. *J Immunol* 2007; **178**: 3822–3830.
49 Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C *et al*. ICOS-dependent extrafollicular helper T cells elicit IgG production *via* IL-21 in systemic autoimmunity. *J Exp Med* 2008; **205**: 2873–2886.
50 Johansson-Lindbom B, Ingvarsson S, Borrebaeck CA. Germinal centers regulate human Th2 development. *J Immunol* 2003; **171**: 1657–1666.
51 Marinova E, Han S, Zheng B. Human germinal center T cells are unique Th cells with high propensity for apoptosis induction. *Int Immunol* 2006; **18**: 1337–1345.
52 Schenka AA, Muller S, Fournie JJ, Capila F, Vassallo J, Delsol G *et al*. $CD4^+$ T cells downregulate Bcl-2 in germinal centers. *J Clin Immunol* 2005; **25**: 224–229.
53 Saito M, Novak U, Piovan E, Basso K, Sumazin P, Schneider C *et al*. BCL6 suppression of BCL2 *via* Miz1 and its disruption in diffuse large B cell lymphoma. *Proc Natl Acad Sci USA* 2009; **106**: 11294–11299.
54 Egle A, Harris AW, Bath ML, O'Reilly L, Cory S. VavP-Bcl2 transgenic mice develop follicular lymphoma preceded by germinal center hyperplasia. *Blood* 2004; **103**: 2276–2283.
55 Lim HW, Hillsamer P, Kim CH. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J Clin Invest* 2004; **114**: 1640–1649.
56 Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L *et al*. Two functional subsets of $FOXP3^+$ regulatory T cells in human thymus and periphery. *Immunity* 2008; **28**: 870–880.
57 Wu HY, Quintana FJ, Weiner HL. Nasal anti-CD3 antibody ameliorates lupus by inducing an IL-10-secreting $CD4^+CD25^-LAP^+$ regulatory T cell and is associated with down-regulation of $IL\text{-}17^+CD4^+ICOS^+CXCR5^+$ follicular helper T cells. *J Immunol* 2008; **181**: 6038–6050.
58 Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T *et al*. Preferential generation of follicular B helper T cells from $Foxp3^+$ T cells in gut Peyer's patches. *Science* 2009; **323**: 1488–1492.
59 Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol* 2006; **6**: 205–217.