# Rethinking peripheral T cell tolerance: checkpoints across a T cell's journey

*Mohamed A. ElTanbouly and Randolph J. Noelle*

Abstract | Following their exit from the thymus, T cells are endowed with potent effector functions but must spare host tissue from harm. The fate of these cells is dictated by a series of checkpoints that regulate the quality and magnitude of T cell-mediated immunity, known as tolerance checkpoints. In this Perspective, we discuss the mediators and networks that control the six main peripheral tolerance checkpoints throughout the life of a T cell: quiescence, ignorance, anergy, exhaustion, senescence and death. At the naive T cell stage, two intrinsic checkpoints that actively maintain tolerance are quiescence and ignorance. In the presence of co-stimulation-deficient T cell activation, anergy is a dominant hallmark that mandates T cell unresponsiveness. When T cells are successfully stimulated and reach the effector stage, exhaustion and senescence can limit excessive inflammation and prevent immunopathology. At every stage of the T cell's journey, cell death exists as a checkpoint to limit clonal expansion and to terminate unrestrained responses. Here, we compare and contrast the T cell tolerance checkpoints and discuss their specific roles, with the aim of providing an integrated view of T cell peripheral tolerance and fate regulation.

Studies over the past 50 years have revealed the remarkable diversity of T lymphocytes with regard to developmental origin, differentiation trajectories, migration and residence patterns, as well as effector, cytotoxic and suppressive activities. With this knowledge came an increased appreciation of the importance of regulatory mechanisms to rein in the potent effector functions of these cells. If not tightly controlled, a single self-reactive T cell can cause crippling damage. The danger of unrestrained T cell responses is manifested in the immune-related pathologies seen in autoimmune diseases and 'cytokine storms' as a consequence of overactive immune responses[1,2]. Multiple layers of negative regulation have been identified during the development and function of T cells, collectively referred to as 'T cell tolerance'. 'Central tolerance' describes the selection mechanisms during thymocyte differentiation that shape the repertoire to limit the survival of self-reactive cells, whereas peripheral tolerance is maintained by multiple mechanisms that restrain peripheral T cell responses to a self-antigen. It has been shown that thymic deletion of self-reactive T cells is only ~60–70% efficient, allowing the peripheral naive T cell repertoire to contain a significant portion of low-avidity, self-reactive T cells[3–5]. These T cells pose the potential risk of autoimmune responses. Therefore, numerous peripheral tolerance checkpoints are critical to regulate the activity and prevent the pathogenicity of these self-reactive T cells, in addition to regulating the magnitude and timing of protective T cell responses to limit overactivity and hyperinflammation in response to pathogens. Peripheral tolerance checkpoints include mechanisms that act directly on the responding T lymphocyte (T cell-intrinsic mechanisms) and T cell-extrinsic mechanisms that depend on other cell subsets, such as regulatory T cells ($T_{reg}$ cells)[6,7] and dendritic cells. Extrinsic tolerance mechanisms have been comprehensively reviewed elsewhere[6,8].

Here, we discuss novel insights into T cell-intrinsic peripheral tolerance mechanisms and discuss their molecular underpinnings. We map the tolerance checkpoints along the journey of a naive T cell from its exit to the periphery to its demise with age. In this effort, we hope to broaden the reader's appreciation of the complexity of peripheral T cell tolerance, with multiple checkpoints utilizing distinct mechanisms to regulate the function of a T cell throughout its lifespan (FIG. 1). We highlight the importance of checkpoints at the naive T cell stage, where T cell quiescence and ignorance may be represented by diverse subsets of naive cells whose phenotypes we have yet to understand. Elaborate mechanisms have been defined after T cell activation, where anergy and exhaustion are two tolerance checkpoints dependent on T cell activation and that play key regulatory roles at the priming and effector stages, respectively. Senescence is an underappreciated tolerance mechanism that is predominant in the terminal effector and memory phases and likely contributes to compromising lymphocyte function with ageing. Lastly, peripheral deletion by different modes of programmed cell death remains a critical point of control in the regulation and termination of T cell responses and is present at virtually every step of T cell differentiation.

## Quiescence

Quiescence is a peripheral tolerance mechanism that can limit the responsiveness of naive T cells to tonic signals. When naive T cells exit the thymus, the quiescence programme, regulated by quiescence mediators, maintains these cells at a lower basal metabolic state than their thymocyte counterparts[9]. Quiescence is an active process that maintains T cells in the G0 stage of the cell cycle, sustains a small cell size, ensures low cellular metabolism and prevents the development of effector functions in response to tonic signals, including self-antigens and other mediators[10–12]. It is an important mechanism to prevent the expansion of the numbers of naive T cells in the steady state and thereby maintain their relative numbers. The mechanisms controlling quiescence also set

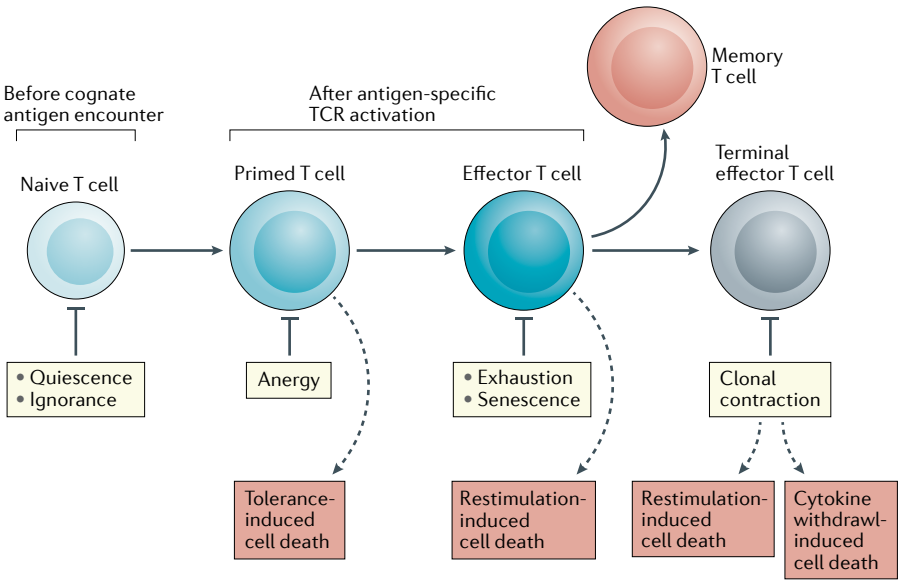


Fig. 1 | **Integrated road map for T cell tolerance checkpoints.** Temporal schematic integrating the tolerance checkpoints at each stage of the peripheral T cell lifespan. Six tolerance checkpoints exist and integrate to regulate T cell responses at all stages. These T cell regulatory checkpoints start at the naive T cell stage, where quiescence and ignorance enforce T cell tolerance. These checkpoints occur before T cell activation by cognate antigen encounter and priming. After antigen-specific T cell activation, co-stimulation-deficient T cell receptor (TCR) signalling can trigger anergy, which enforces T cell hyporesponsiveness and limits T cell responses to inappropriate stimuli (such as self-antigens). Such tolerogenic activation can also induce peripheral T cell deletion, known as tolerance-induced cell death. As a result of chronic antigen stimulation during the effector T cell stage, exhaustion and senescence can limit T cell responses. During the effector stage and beyond (where terminal effector T cells undergo clonal contraction), restimulation-induced cell death and cytokine withdrawal-induced cell death serve to terminate T cell responses.

the threshold for activation of naive T cells. As such, quiescence plays a critical role in clonal selection, ensuring that most of the repertoire that binds a particular antigen is restrained and limiting clonal expansion to high-affinity clones. However, it is unclear whether the threshold for quiescence is fixed or exists within a dynamic spectrum. It is possible that the mediators of quiescence can be downregulated to allow naive T cell responses to lower-affinity antigens or upregulated to restrict responses more stringently. Given that the signalling molecules and networks that control T cell quiescence are dynamic and respond to outside cues, one may surmise that the threshold for quiescence changes in different environmental contexts and that these changes determine the set point for a productive response to an antigen, the development of autoimmunity or the response to neoantigens in cancer.

In mice, many of the molecular effectors and networks involved in T cell quiescence have been identified and have been shown to control a diverse array of biological pathways. TGFβ1 and TOB1, two effectors that are part of the transforming growth factor-β superfamily signalling network, have been identified as quiescence mediators[13–15]. They signal via the SMAD family of signal transducers and downregulate the production of IL-2 and T cell activation in general. *TOB1* is a member of the APRO family of genes, which encodes proteins with antiproliferative functions and also includes *BTG1* and *BTG2* (REF.[16]). Recently, it was shown that all these genes are selectively and highly expressed in naive T cells and that BTG1 and BGT2 play an important role in promoting the deadenylation and degradation of mRNA in naive T cells, thereby actively maintaining a low rate of translation[17,18].

Transcriptional and proteomic analyses of the naive T cell repertoire have also revealed a high expression of the DNA-binding protein KLF2 by quiescent T cells, qualifying it as a marker for T cell quiescence[17,19]. KLF2 impacts on T cell quiescence partly through the suppression of the transcription factor MYC and by affecting cell cycle progression through p21 (REFS[20–23]). KLF2 also appears to regulate thymocyte egress and the expression of S1PR1 and CD62L, two receptors that are highly expressed by naive T cells[24,25]. Whether this lymphocyte recirculation regulatory mechanism by KLF2 plays an important role in the maintenance of quiescence remains to be determined. In T cells, the expression of KLF2 is induced and maintained by the transcription factor FOXO1, which has a well-established role in maintaining T cell quiescence[26–28]. Indeed, the expression of FOXO1 and KLF2, as well as the expression of the transcription factor FOXP1, are all directly upregulated by the transcription factor RUNX1, which also has an important regulatory role in maintaining T cell quiescence, as evidenced by the observation that loss of RUNX1 leads to T cell hyperactivation[29,30]. As mentioned above, part of the quiescence phenotype is a low basal metabolic programme. The tumour suppressor proteins TSC1 and TSC2 play an important role in this process by suppressing the activation of mechanistic target of rapamycin complex 1 (mTORC1), an important nutrient, energy and redox sensor[31,32]. This results in a downregulation of cellular metabolism, thereby maintaining low nutrient demands by the large number of peripheral naive T cells.

Insights into how these interconnected, multidimensional networks of quiescence regulators control the threshold of responsiveness of naive T cells in health and disease are evolving[33]. For all documented quiescence regulators (TABLE 1), their genetic deletion results in an increased percentage of T cells with a memory phenotype, with enhanced activation and pro-inflammatory polarization states[17,22,27,32]. Moreover, there is ample evidence that alterations in the expression of quiescence regulators result in a breakdown in peripheral tolerance and the development of autoimmune disease[28,30,34]. For example, several studies have shown that abrogation of TGFβ1 signalling in T cells precipitates aggressive and fatal autoimmune disease[35,36], likely due to disruption of the suppressive activities of SMAD and TOB1 on T cell activation. In addition, *Foxo1* deletion in T cells in mice results in the development of profound colitis, pancreatitis and multi-organ lymphocyte infiltration; however, this may be partly caused by $T_{reg}$ cell dysfunction in addition to lowering of the threshold of naive T cell quiescence. Mice with *Runx1*$^{-/-}$ T cells experience a cytokine-release syndrome and fatal pneumonitis[30]. Collectively, these findings suggest that a breakdown of T cell quiescence lowers the threshold of naive T cell responsiveness to self-antigens, resulting in profound autoimmunity.

Recent studies have established that the naive T cell repertoire in mice (defined by CD62L$^{hi}$ and CD44$^{low}$) is not uniform

or monomorphic but displays remarkable steady-state heterogeneity with multiple cellular subsets[17]. The role of specific networks of quiescence mediators in controlling the steady-state distribution of these subsets is not currently understood. Whereas most naive T cells exhibit a quiescent phenotype and coexpress high levels of genes encoding quiescence regulators, there are several subsets within the naive T cell compartment[17], including T cells with high levels of expression of genes encoding the type I interferon response module, others with enhanced early T cell receptor (TCR) signalling activity and subsets with augmented expression of genes encoding cytoskeleton components[17]. We argue that the current phenotypic definition of naive T cells needs to be expanded beyond the use of conventional markers such as CD44, CD62L and CD45RB to those that are more relevant to quiescence such as KLF2 and TOB1 to more appropriately capture the heterogeneous landscape within the naive repertoire.

Importantly, it appears that the expression of the inhibitory checkpoint receptor VISTA is critical for the intrinsic, steady-state maintenance of these heterogeneous naive T cell subsets and for the maintenance of quiescence[17,37]. It was shown that *VISTA*$^{-/-}$ T cells (*VISTA* is also known as *VSIR*) express reduced levels of important quiescence mediators such as KLF2, BTG1 and BTG2 (REF.[17]). Further analysis revealed that the expression of VISTA and KLF2 is co-regulated in T cells and suggests a direct relationship between VISTA expression and the maintenance of quiescence networks. The loss of key quiescence regulators in *VISTA*$^{-/-}$ T cells results in hyper-responsiveness to co-stimulation-deficient T cell activation and precipitates several autoimmune manifestations, including experimental autoimmume encephalomyelitis, splenomegaly and enhanced infiltration of activated T cells into non-lymphoid tissues in mice[38–40]. These findings link tonic VISTA signalling, elements of the quiescence networks and the prevention of autoimmunity.

At present, the steady-state heterogeneity of the human naive T cell compartment is not well explored[41]. There are indications that some factors that are critical for maintaining mouse T cell quiescence and their naive phenotype are functionally conserved across mouse and human T cells. For example, it has been reported that TOB1 and KLF2 are quiescence regulators that are common to both mouse and human T cells[42,43]. A recent study showed that the same transcription factors implicated in naive T cell quiescence in mice (FOXO1, FOXP1 and KLF2) are also highly expressed in human naive T cells[44]. These transcription factors have a constitutively rapid turnover, enabling their depletion on T cell activation to facilitate the transition from a quiescence state to effector T cell ($T_{eff}$ cell) differentiation[44]. Although we map quiescence as a tolerance checkpoint to naive T cells, there are also indications that memory T cells exhibit quiescence features (discussed in BOX 1).

## Ignorance

Ignorance is another tolerance checkpoint at the naive T cell stage that has been observed in multiple systems, yet its mechanisms remain poorly understood[45]. Simply put, self-reactive T cells can fail to activate and provoke autoimmune disease despite the presence of the specific self-antigen. These T cells remain in a naive, responsive state. Mechanisms that control ignorance can include intrinsic and extrinsic mechanisms. Intrinsic mechanisms include TCR affinity for an antigen where TCR affinity is too low to elicit a T cell response. Extrinsic mechanisms likely comprise lack of T cell stimulation owing to low antigen density and/or the restriction of antigen recognition owing to its anatomical location[46]. This was demonstrated in two recent studies using elegant mouse models of neo-self-antigen expression restricted to specific organs based on tissue-specific promoters, which allowed the tracking of the response of antigen-specific endogenous T cells in vivo[47,48]. In both cases, the investigators identified 'ignorance' of a self-antigen as the main mechanism curtailing reactivity and immunopathology to an antigen expressed in the pancreas but not in the lung or intestines, where thymically derived $T_{reg}$ cells instead emerged[47,48]. They concluded that the anatomical location and abundance of the self-antigen are the deciding factors, as low antigen dose in the pancreas maintained ignorance by not provoking T cell priming, whereas high doses elsewhere resulted in deletional peripheral tolerance[49].

Table 1 | **Summary of the main regulators and markers of each tolerance checkpoint in T cells**

| Factor | Quiescence | Ignorance | Anergy | Exhaustion | Senescence | Deletional tolerance |
|---|---|---|---|---|---|---|
| Surface receptors | TGFβR1 (REF.[13])<br>VISTA[17] | Unknown | CD73 (REFS[78,79])<br>FR4 (REFS[78,79])<br>LAG3 (REF.[85])<br>NRP1 (REF.[79]) | PD1 (REF.[177])<br>TIGIT[178]<br>LAG3 (REF.[179])<br>TIM3 (REF.[180]) | NKG2D[133]<br>IFNα/IFNAR[119] | FAS (also known as CD95)[160,161]<br>TNFR1(REFS[164,181])<br>TRAILR1 and TRAILR2 (REFS[182,183]) |
| Signalling molecules | BTG1/BTG2 (REF.[18])<br>TSC1/TSC2 (REFS[31,32]) | Unknown | DGKα[62]<br>CBLB[67]<br>GRAIL[184]<br>ITCH[185,186] | SHP1 (REF.[187])<br>SHP2 (REFS[187,188])<br>PTPN2 (REF.[189]) | TAB1 (REF.[129])<br>Sestrin 2 (REF.[133]) | CASP8 (REF.[161])<br>BID[190]<br>BIM (also known as BCL-2L11)[145,146] |
| Transcription factors | KLF2 (REFS[20,22])<br>FOXO1 (REFS[26–28])<br>RUNX1 (REFS[29,30])<br>TOB1 (REFS[14,15])<br>FOXP1 (REFS[191,192]) | Unknown | NFAT1 (REF.[64])<br>EGR2 (REFS[66–68])<br>EGR3 (REF.[67])<br>NR4A1 (REF.[72])<br>TOB1 (REF.[15]) | IRF4 (REF.[193])<br>NR4A1 (REF.[103])<br>GATA3 (REF.[194])<br>TOX[103–106]<br>BATF[102]<br>BLIMP1 (REF.[195])<br>EOMES[196] | – | – |

DGKα, diacylglycerol kinase-α; IFNα, interferon-α.

Box 1 | **Is quiescence a tolerance mechanism in memory T cells?**

It is tempting to speculate that memory T cells, in the absence of a cognate antigen, can enter a quiescent stage that is similar to quiescence observed in naive T cells, although recent evidence indicates that memory T cells can have complex and heterogeneous phenotypes. Indeed, central memory T cells sustain a low basal metabolism and have a slow turnover. This is critical for the longevity and 'stemness' of these memory T cells and allows the maintenance of long-term immunity to particular pathogens. Despite these features, there are clear distinctions between naive quiescent T cells and memory T cells that appear quiescent, as well as differences within the memory T cell subsets with regard to the levels of quiescence they exhibit. Therefore, the factors that define quiescence in naive T cells are different from those in memory T cells. Unlike their naive counterparts, memory T cells are characterized by basal proliferation driven by homeostatic cytokines (for example, IL-7 and IL-15)[197] and are always kept in a state of 'readiness'[11] via two known mechanisms. First, most memory T cells are actively[198] maintained in the G1 state, whereas naive T cells are maintained in the G0 state[198,199]. This endows memory T cells with the potential for rapid proliferative and functional recall responses. Second, memory T cells exhibit markedly enhanced chromatin accessibility to genes encoding important effector functions[200], which largely accounts for their efficient recall responses to antigens, and a lower threshold for antigen responsiveness. In the particular case of tissue-resident memory T cells, several studies showed a marked reduction of expression of quiescent mediators and an enhancement in the expression of T cell receptor downstream effectors and cytokine genes compared with other memory subsets and to naive T cells[201,202], keeping the tissue-resident memory T cell subset "'frozen' in a near-effector status"[203].

A significant observation from these studies is that self-reactive ignorant T cells do exist in the periphery of these mice[47,49–51]. Similarly, ignorant self-reactive T cells exist in human peripheral blood and can be as frequent in healthy individuals as in patients with autoimmune disease. The difference is that self-reactive T cells in healthy individuals are maintained in a naive state, whereas these T cells have an activated phenotype in individuals with autoimmune conditions[52–54]. In light of the substantial steady-state heterogeneity now known to exist within the naive T cell compartment, a reassessment of T cell ignorance in humans and mice at much higher resolution is warranted. Studies are currently under way to evaluate whether naive, ignorant self-reactive T cells may differ with regard to their quiescence mediators, as highly resolved phenotypic analyses (for example, single-cell RNA sequencing) have yet to be reported.

A noteworthy distinction between quiescence and ignorance is that quiescence is a general tolerance hallmark of all naive T cells, irrespective of their antigen specificity, whereas 'ignorance' refers to the avoidance of activation of specific self-reactive T cells, with the host tissue and the location of the self-antigen being determining factors. Much more is understood about the regulators of naive T cell quiescence than the molecular regulators of T cell ignorance. It is currently unknown whether some of these regulators overlap.

Like quiescent T cells, and unlike anergic T cells as discussed later, 'ignorant T cells' are not dysfunctional, as when appropriately stimulated in an inflammatory context, such as with viral pathogens[50,51] or inflammatory cytokines[55,56], they are capable of overriding the ignorance checkpoint and inducing autoimmune disease[57,58].

One of the unanswered questions regarding T cell ignorance is whether there is an evolutionary benefit to imperfect central tolerance that results in a significant number of ignorant self-reactive T cells reaching the periphery. Mouse models in which central tolerance is titrated to eliminate all self-reactive clones may be of value here. Despite the lack of empirical investigation, we speculate that these low-avidity self-reactive T cells are likely reactive and may have high specificity for some pathogens and that their potential protective benefit in an infection setting may outweigh the risk they pose with regard to autoimmunity.

## Anergy

The mechanisms of quiescence, ignorance and anergy all serve to limit the responses of naive T cells to an antigen. However, quiescence is constitutively maintained in a manner that is agnostic to TCR stimulation, ignorance is a result of the antigen being hidden away or presented at extremely low levels, whereas anergy is a direct result of 'defective (imbalanced)' TCR stimulation in naive T cells. Anergy is the most proximal, non-deletional tolerance mechanism following TCR engagement and is functionally defined as T cell hyporesponsiveness to restimulation under robust stimulatory conditions. Functionally, it serves as an early checkpoint during T cell priming to prevent potential T cell pathogenicity before the onset of the T cell effector stage.

At the molecular level, anergy is induced by co-stimulation-deficient ('tolerogenic') TCR activation. In T cells that receive TCR signalling with productive co-stimulation (for example, CD28), this induces the transcription factor NFAT1 together with AP-1 (a dimeric transcription factor composed of FOS and JUN family subunits) to induce T cell differentiation and effector functions. By contrast, tolerogenic TCR activation leads to a defect in RAS–mitogen-activated protein kinase (MAPK) signalling[59–62], which in turn impairs translocation of AP-1 into the nucleus[63]. In this instance, TCR signalling is now shifted towards the transcription factor NFAT1 in the absence of AP-1 activity. This imbalanced shift in downstream TCR signalling towards NFAT1 activation results in the induction of several genes that encode proteins involved in establishing the anergic state[64], such as diacylglycerol kinase-α (DGKα)[64], an enzyme critical for anergy induction through inhibition of RAS activation via depletion of diacylglycerol[62,65]. Immediate transcriptional targets of NFAT1 also include the transcription factors EGR2 and EGR3, which suppress IL-2 transcription and upregulate the regulatory ubiquitin ligase CBLB[66–68].

The long-term TCR-induced hyporesponsive state observed in anergic T cells shows substantial evidence of coordinated epigenetic[69,70] and post-transcriptional[71] programming that silences effector cytokine expression. Recent work identified the TCR-induced gene *Nr4a1* (also known as *Nur77*), which encodes a nuclear receptor that acts as an inhibitor of AP-1 function, to mediate the epigenetic reprogramming of mouse T cells towards an anergic state[72]. Hallmark functional changes that define anergy include a profound reduction in the levels of IL-2, interferon-γ and tumour necrosis factor (TNF) in response to TCR stimulation. This acquired refractory state is associated with growth arrest and defects in cell cycle progression. Although anergy can be long-lasting, it is reversible, and in vivo studies show that anergic T cells slowly recover functional responsiveness in the absence of the antigen, indicating that the maintenance of anergy requires prolonged antigen exposure[73–75]. The reversal of anergy is also observed on adoptive transfer of anergic cells into a lymphopenic environment. In this case, the absence of a cognate antigen and the abundance of homeostatic cytokines result in the establishment of an effector cell state[76,77].

It appears that TCR stimulation of quiescent T cells results in the downregulation of factors that enforce quiescence, whereas TCR stimulation of anergic T cells upregulates mediators of anergy. Transcriptional profiles of naturally anergic CD4$^+$ T cells (defined as CD44$^{hi}$CD73$^{hi}$FR4$^{hi}$FOXP3$^-$)[78] revealed that, following TCR engagement, most quiescence regulators, with the exception of TOB1 (REF.[15]), are downregulated, whereas several factors involved in the acquisition of the anergic state, including MAF, NFAT1, NRP1 and NR4A1, are upregulated[17,79,80]. One key similarity between quiescent and anergic T cells is that mTORC1 activity is suppressed in both states. The metabolic consequences of mTORC1 suppression include reduced protein synthesis, as well as nutrient acquisition via suppressed expression of amino acid and glucose transporters, all key features for the energy charge necessary for the acquisition of T cell effector functions[81,82]. In anergic T cells, the absence of co-stimulation prevents the full mobilization and upregulation of the metabolic machinery, even by subsequent productive stimuli[82,83]. However, a striking difference between quiescent and anergic T cells is that the former are maintained in the non-proliferative G0 stage of the cell cycle, whereas the induction of anergy involves early proliferation, which is followed by an arrest of cell cycle progression in the G1 to S phase[84]. Thus, there are substantial differences but also similarities in the tolerance mechanisms of naive T cells before and after T cell activation.

Distinctive markers for anergy across T cell subsets and across species have yet to be clearly identified, and most studies resort to an operational definition (a state of tolerance induced by defective TCR stimulation) to classify T cells as anergic. In mice, recent studies defined anergic CD4$^+$ T cells by their higher expression of the 5′ nucleotidase CD73 and the folate receptor (FR4) than $T_{eff}$ cells, and they are distinguished from $T_{reg}$ cells by their lack of FOXP3 expression[77,78]. Whether CD73 and FR4 are functionally involved in the induction or maintenance of anergy, and whether these are exclusive markers of CD4$^+$ anergic cells, remains to be determined. In several mouse tumour models, anergic CD8$^+$ T cells were found to express the inhibitory receptors LAG3 and 4-1BB, which are both regulated by the transcription factor EGR2 (REF.[85]). It is currently unknown whether human anergic T cells display similar molecular markers. Human anergic CD8$^+$ T cells have been defined as expressing both the co-inhibitory receptor CTLA4 and the chemokine receptor CCR7, which are not co-expressed by activated or naive T cells. Surprisingly, these studies reported no differences in the expression of the inhibitory receptor PD1 or the anergy-related genes *EGR2*, *GRAIL* (also known as *RNF128*) and *CBLB*[86]. Efforts are ongoing to establish the common phenotypes that define anergic T cells in both mouse and human T cell subsets and to examine the role of anergy in anticancer immune response (BOX 2).

## Exhaustion

T cell exhaustion represents the predominant non-deletional tolerance mechanism at the T cell effector stage. The term 'T cell exhaustion' was coined by viral immunologists who described desensitized T cells in the context of chronic viral infections[87,88]. During an acute immune response such as the response to an acute infection, functional central memory T cell ($T_{CM}$ cell) and effector memory T cell ($T_{EM}$ cell) subsets arise from $T_{eff}$ cells. By contrast, when antigen stimulation persists, such as during chronic infection and in certain types of cancer, memory T cells can fail to develop properly[89–91], and functionally compromised, persistently 'exhausted' T cells ($T_{ex}$ cells) dominate the antigen-specific repertoire. Compared with fully functional primed $T_{eff}$ cells, $T_{ex}$ cells display reduced but not absent responses to antigens at multiple levels. Characteristic features of $T_{ex}$ cells include (1) decreased cytokine production, (2) sustained, high levels of inhibitory receptor expression, (3) altered epigenetic, metabolic and transcriptional states and, importantly, (4) an inability to transition to the quiescence-like cell state observed in memory T cells (BOX 1). However, recent findings suggest that there may be differences in the $T_{ex}$ cell state in chronic viral infections and in cancer (BOX 3).

Initial studies of the $T_{ex}$ cell phenotype found that these cells express multiple inhibitory receptors, including PD1, LAG3, TIGIT, CD38, CD39 and TIM3 (REF.[92]). Indeed, subsequent work defined a stage of T cell exhaustion based on these markers[93]. However, we now know that these receptors are not exclusive features of $T_{ex}$ cells as (1) highly functional $T_{eff}$ cells can also express inhibitory receptors and mediators of exhaustion such as TOX[94–96], (2) human T cells express some of these markers in the steady state and (3) their expression dynamically varies according to localization and differentiation state[97,98]. Another challenge is the difficulty in distinguishing $T_{ex}$ and anergic T cells on the basis of surface markers as these two states of T cell dysfunction overlap with regard to the expression of several of the inhibitory receptors. TCR stimulation is the central driver of T cell hyporesponsiveness and the resultant dysfunctional state for both $T_{ex}$ cells and anergic T cells. However, the key distinction between anergy and exhaustion is that anergic T cells arise early after T cell activation and priming, whereas $T_{ex}$ cells arise from $T_{eff}$ cells that

### Box 2 | Does T cell anergy limit antitumour immune responses?

Although anergy is an early and effective negative regulator of T cell activity, its potential role in modulating the immune response in human disease requires further elucidation. Anergy is a tolerogenic mechanism of unresponsiveness that occurs during the priming stage, early after naive T cell activation (as opposed to exhaustion, which occurs after the initial productive stimulation and the acquisition of effector functions). There has been an intense focus on the role of T cell exhaustion in the tumour microenvironment, and there is evidence that dysfunctional T cells in the tumour microenvironment comprise both exhausted T cells and anergic T cells. First, most cancer types lack dominant immunogenic features because they do not express co-stimulatory molecules or inflammatory cytokines, as are generally present during viral or bacterial challenges[204–207]. As expected, this suboptimal priming of T cells in the tumour microenvironment can lead to anergy in tumour-specific T cells[208–210]. Second, the profound state of T cell dysfunction in tumour models appears very early after T cell activation, indicating that a substantial number of T cells might be anergic rather than exhausted[211,212]. Third, it has been shown that T cell dysfunction in tumours can be overcome through blockade of CTLA4 (REFS[213,214]) or LAG3 (REF.[85]), or agonism of OX40 (REF.[215]). These checkpoint molecules determine the outcome of T cell priming, indicating that T cell dysfunction in tumours may be partly due to the presence of anergic T cells. Finally, a recent study has shown that tumour-specific CD4$^+$ T cells that are activated and proliferate in the tumour-draining lymph node become anergic and ultimately differentiate into peripheral regulatory cells[216]. Understanding the role of anergic T cells in tumour immunity is critical as recent in vivo findings suggest that PD1 blockade of subprimed (primed under conditions lacking optimal co-stimulation) anergic T cells can lead to worse therapeutic outcomes by worsening pre-existing T cell dysfunction[212]. Clearly, a comprehensive appreciation of the breadth of CD4$^+$ (and CD8$^+$) T cell anergy and the impact of checkpoint blockade on this process will lead to better therapeutic strategies to enhance tumour immunity.

Box 3 | **Are there differences in T cell exhaustion in chronic infections and in cancer?**

Recent findings suggest that there is a significant difference between T cell exhaustion in chronic viral infections versus cancer[217,218]. Although there are multiple phenotypic and molecular features of exhausted T cells ($T_{ex}$ cells) that are shared between both systems, including inhibitory receptor expression, $T_{ex}$ cells in tumour models often present with a profound defect in effector functions and an inability to control tumour growth and metastasis compared with chronic viral infection, where $T_{ex}$ cells show functional responses impeding lethality[219]. Second, tumour-specific $T_{ex}$ cells arise early after tumorigenesis[220], and there is a clear consensus that most tumours cause suboptimal priming of T cells[212,220,221], unlike most viral infection models, where appropriate T cell stimulation occurs initially. Earlier work using models of lymphocytic choriomeningitis virus infection suggested that blocking the PD1 pathway can reduce viral load[177]. However, molecular studies of the epigenetic state of $T_{ex}$ cells after PD1 pathway blockade showed a distinct inflexible epigenetic profile, which caused these $T_{ex}$ cells to sustain their dysfunction[222]. This study suggested that PD1 pathway blockade induces a transient transcriptional rewiring in the $T_{ex}$ cells that allows them to better engage modules of effector genes[222]. More recent work using single-cell T cell receptor sequencing in tumour systems showed that the clonality of the tumour-infiltrating cells after PD1 blockade did not match the $T_{ex}$ cell population, but the tumour-infiltrating cells were of novel clonotypes that had not existed in the same tumour[223]. This suggests that blocking the PD1 pathway does not significantly impact the tumour-infiltrating $T_{ex}$ cell population but rather prevents the exhaustion of newly generated tumour-specific effector T cells. Of equal importance, T cell anergy in the tumour microenvironment (see BOX 2) can markedly undermine this favourable impact of PD1 blockade on host defence[212].

have undergone productive activation, at the effector (memory-precursor) stage[99]. Moreover, the natures of the signals that induce these cell stages differ. Anergy is the product of co-stimulation-deficient T cell activation, whereas exhaustion occurs owing to chronic TCR stimulation in the presence of appropriate co-stimulatory signals.

On the molecular level, both anergic T cells and $T_{ex}$ cells express NFAT1 as an important driver of tolerance. However, the gene expression profile typical for $T_{ex}$ cells also appears to be determined by a complex transcription factor profile that includes reduced expression of TCF7 and increased expression of TOX, NR4A, BATF, IRF4, BLIMP1 and other transcription factors[100,101]. High-throughput transcriptional and epigenetic analyses identified several transcription factors induced by TCR stimulation that are involved in the induction of the $T_{ex}$ cell state and in allowing the $T_{ex}$ cells to survive beyond the $T_{eff}$ cell contraction phase. These transcription factors include NFAT, IRF4, BATF[102], NR4A1 (REF.[103]) and TOX[103–106]. However, an important observation from these studies is the identification of a 'progenitor' or 'precursor' population of predysfunctional $T_{ex}$ cells that have self-renewal (stem cell-like) properties and re-expansion potential and are defined by the expression of the transcription factor TCF1 (also known as TCF7)[107–109]. Of note, this cell state is still inferior in effector function in comparison with fully functional $T_{eff}$ cells but is likely responsive to checkpoint blockade[108]. With prolonged activation, these progenitor $T_{ex}$ cells ultimately give rise to the terminally differentiated dysfunctional subset called 'terminal $T_{ex}$ cells'.

Some consensus and controversies exist in defining the mediators and markers of exhaustion in mouse and human T cells. In both mice and humans, TCF7 has emerged as a transcription factor critical for defining the progenitor subset of self-renewing T cells that can re-expand even in settings of chronic infection or cancer[110]. A $T_{ex}$ cell immune signature predicted favourable prognosis in multiple human autoimmune and inflammatory diseases[111]. In transplantation, kidney transplantation from a CMV-positive donor into a CMV-negative recipient leads to an $T_{ex}$ cell state that may play a role in graft tolerance[112]. This is supported by mouse models where a $T_{ex}$ cell state is correlated with reduced graft rejection of heart and liver transplants[113–115]. Despite these similarities, there remains a great deal of mechanistic investigation to understand human T cell exhaustion.

Our understanding of T cell tolerance suggests that T cell exhaustion has several evolutionary benefits. From a systems perspective, $T_{ex}$ cells persist beyond the $T_{eff}$ cell lifespan and contribute to the containment of chronic viral infections. Second, exhaustion permits pathogen-specific T cells to curtail their activity to avoid persistent inflammation, tissue damage and chronic autoreactivity. This is important for host survival, as failure in the induction of exhaustion[113–115] or reinvigoration of $T_{ex}$ cells in some viral models can induce immunopathology[116,117].

### Senescence

Senescence is defined as a growth and proliferative arrest stage that is induced when "cells reach the end of their replicative potential or are exposed to various stressors"[118]. As discussed already, chronic antigen stimulation can cause the functional exhaustion of $T_{eff}$ cells. However, repeated TCR stimulation or lymphocyte ageing can also induce telomere erosion and/or irreparable DNA damage, leading to a loss of the T cell replicative capacity on further antigen encounters, which occurs at the effector or memory stage of T cell differentiation. Telomere-dependent senescence occurs during extensive replication and can be the result of repeated clonal expansion and antigen recall (where pre-existing memory T cells respond to previously encountered antigens). However, it is now known that there are factors that can inhibit telomerase and accelerate senescence, such as interferon-α, which directly inhibits telomerase activity[119]. On the other hand, telomere-independent senescence can be induced by DNA-damaging agents such as reactive oxygen species (ROS) and ionizing radiation or by activation of the p53 pathway or other stress pathways in response to growth factor deprivation[120]. Strong TCR stimulation and oxidative phosphorylation as a result of high-affinity antigen encounter can enhance the production of ROS[121,122], which can affect T cell function and induce cell death[123]. However, little is known about the role of telomere-independent senescence in T cell tolerance.

Studies on primary human T cells identified senescent T cells as poorly proliferative highly differentiated T cells that display the surface markers CD45RA, KLRG1 and CD57 but do not express the co-stimulatory receptors CD27 and CD28 (REF.[124]). Whereas $CD27^+CD28^+$ T cells have long telomeres, senescent T cells with the shortest telomeres lose expression of CD27 and CD28 but re-express CD45RA[124–126]. Unlike $T_{ex}$ cells, senescent T cells are not compromised with regard to effector function but acquire a senescence-associated secretory phenotype that is characterized by the production of high levels of pro-inflammatory and suppressive cytokines, despite their proliferative block[124,125,127]. Senescent T cells have constitutively activated p38 MAPK activity, and the inhibition of this activity can reconstitute proliferation and telomerase activity[128]. However, this constitutive p38 activity is not mediated by the canonical pathway of p38 activation through upstream

MAPK, or the alternative pathway of p38 activation induced by the TCR, but rather is mediated through the intracellular metabolic sensor AMPK, which triggers p38 autophosphorylation via the scaffold protein TAB1 (REF.[129]). These findings suggest that senescence is actively maintained in T cells, as has been shown for other cell types. They also implicate DNA damage and nutrient sensing in the induction of T cell senescence.

Senescent T cells markedly accumulate with ageing, during chronic viral infections, in individuals with autoimmune disorders and in individuals with particular types of cancer[125,130–132]. From an evolutionary standpoint, senescence may protect against T cell lymphoma development by preventing the excessive proliferation of T cells with damaged DNA. Another benefit may be the local control of excessive inflammation during chronic autoimmunity or infections. It is unclear whether T cell senescence has any benefit during ageing, where instead it appears detrimental to T cell responses and productive immunity. Recently, a study showed that senescent $CD8^+$ T cells lost TCR signalling in aged individuals and were reprogrammed to acquire an innate-like cytotoxic activity via the upregulation of the natural killer cell receptor NKG2D pathway. This $CD8^+$ T cell to natural killer cell-like transformation was mediated via the stress-sensing protein Sestrin 2 (REF.[133]). However, T cell senescence can also lead to a loss of immune functions. For example, reports show a loss of memory T cell proliferative responses to antigens in senescent T cells, and the loss of clonal expansion in response to antigenic rechallenge[134]. These observations are important in the setting of vaccination of elderly people, where senescence may hamper the clonal expansion of T cells necessary for primary immune responses[134–136].

One major challenge in advancing our understanding of T cell senescence is the almost complete absence of studies of T cell senescence in mouse models. This limits our knowledge of how reversible senescence is and whether therapeutic interventions to reverse senescence in T cells can enhance T cell memory and immunity. The senescence tolerance checkpoint may well become increasingly relevant as human life expectancy is increasing[137], given that ageing individuals develop enhanced susceptibility to infections to which they were previously immune[134,138]. Immunity clearly diminishes with age, and a major factor in this decline is compromised T cell function[139–141]. Therefore, more mechanistic in vivo studies in multiple settings are needed to qualify and illuminate the relevance and setting of this tolerance checkpoint.

**Peripheral deletional tolerance (death)**

Multiple programmed cell death checkpoints exist at several stages of the T cell's journey. However, the signalling cues that induce death in T cells vary according to stage of differentiation. Overall, deletional tolerance serves a central role in pruning the repertoire of peripheral T cells as well as in terminating the immune response after a productive immune challenge (such as during the contraction phase). However, unlike the other cell-intrinsic mechanisms of tolerance discussed in previous sections, this mechanism irrevocably eliminates clones from the repertoire. Although the role of clonal deletion in central tolerance is well studied, the role of T cell death in maintaining peripheral tolerance has been largely overlooked.

Co-stimulation-deficient T cell activation by either an antigen or a superantigen elicits naive T cell activation, limited proliferation anergy and/or death[73,142]. The precise conditions that result in the induction of anergy versus death under co-stimulation-deficient conditions are yet to be resolved. However, after the initial phase of T cell activation and proliferation in response to a tolerizing stimulus (co-stimulation-deficient antigen), a large proportion of T cells are rapidly lost by apoptosis, and the surviving minor population develop an anergic profile[73,143,144]. It has been presumed that the sole engagement of the TCR (signal 1) in the absence of co-stimulation (signal 2) governs the induction of cell death. However, recent studies have shown that genetic deficiency of VISTA on resting T cells spares T cells from TCR-induced death but does not affect the induction of anergy, whereas triggering through VISTA enhances tolerogen-induced death[17]. Therefore, there may be factors, in addition to TCR engagement by antigens, that regulate the magnitude of tolerogen-induced T cell death in the absence of co-stimulation.

A series of elegant studies in mouse models have defined the molecular aspects of death induced by a tolerogen of naive T cells. These studies demonstrated that cell death was mediated via the intrinsic proapoptotic family member BIM[145,146], which is a distinct pathway from the extrinsic pathway of apoptosis mediated by death receptors such as FAS. Of importance, these findings support earlier work highlighting how co-stimulation via CD28 or γC cytokines (IL-7 and IL-4) augments T cell survival after activation via the upregulation of the antiapoptotic factors BCL-$X_L$ (REFS[147–149]) and BCL-2, respectively[150,151]. Highly resolved transcriptional profiling of mouse T cells under tolerizing versus immunizing conditions revealed a unique molecular signature of cells before apoptosis. This included the upregulation of genes such as *Rankl* (also known as *Tnfsf11*), *Bim* (also known as *Bcl2l11*) and the Nr4a family members *Nr4a1*, *Nr4a2* and *Nr4a3*, coupled with the downregulation of the cytokine receptor IL-7Rα[152], all changes that can lead to T cell death[153,154]. Of note, the expression and role of NR4A1 in multiple tolerance checkpoints (anergy[72], exhaustion[103] and death[152]) warrants further studies that elucidate the contribution of this regulator to each of these settings.

An important mechanistic finding was that the balance between the proapoptotic mediator BIM and the antiapoptotic mediator BCL-2 can determine the eventual fate of the tolerized T cell. It was shown in a model of antigen expression and tolerization of transgenic antigen-specific T cells that BIM-deficient T cells are resistant to deletion in response to tolerogenic stimulation but become equally anergic to their wild-type counterparts[155]. Additional studies are needed to define the exact mechanisms that lead tolerogenic stimulation to drive peripheral deletion versus anergy.

A second peripheral death checkpoint occurs when activated $T_{eff}$ cells are restimulated after activation, leading to activation-induced cell death (AICD)[156,157] (more recently described as restimulation-induced cell death[158]). This cell death mechanism is induced by the extrinsic pathway of apoptosis via death receptors (the most well described is FAS (also known as CD95)[159,160], but they also include TNFR1, TRAILR1 and TRAILR2[161]), which signal through caspase 8 to trigger downstream executioner caspases. The intriguing finding that mouse and human $CD4^+$ T cells are selectively susceptible to FAS-mediated AICD[162,163] whereas human $CD8^+$ are more susceptible to TNFR1-mediated AICD[164] is noteworthy and warrants further investigation. This peripheral deletion mechanism serves as a self-limiting feedback process to control T cell expansion and is essential for the process of clonal contraction, wherein antigen-specific $T_{eff}$ cells are eventually eliminated during the termination of an immune response. Co-stimulation is important in this context because signalling through

CD28 helps in reducing AICD in T cells in the clonal expansion phase through the strong upregulation of the caspase 8 inhibitor cFLIP[165] and the upregulation of antiapoptotic factor BCL-$X_L$ (REF.[147]). T cells are sensitive to FAS-induced AICD only after BCL-$X_L$ downregulation, which occurs later in the effector response. Therefore, T cells in the initial clonal expansion phase are spared from FAS-induced deletion by AICD. At present it is unclear how memory T cells survive this clonal contraction phase, although it is thought that their dependence on homeostatic cytokines and the upregulation of prosurvival molecules may play a central role[160]. For example, effector cells destined for memory cell differentiation exhibit higher IL-7R expression, although enforced IL-7R expression does not skew the cells towards a memory fate[166]. It has also been demonstrated that the sensitivity to IL-2 can determine the survival of these T cells, as IL-2RA$^{hi}$ $T_{eff}$ cells are more prone to apoptosis, whereas less-sensitive IL2RA$^{low}$ cells give rise to long-lived memory T cells[167]. The findings regarding IL-2 may appear counterintuitive at first glance but are appreciated through examination of the dual role of IL-2 in T cell survival at different stages. During the initial phase of T cell activation, IL-2 promotes survival and clonal expansion. By contrast, during the down phase (clonal contraction at the end of a response), IL-2 promotes sensitization to restimulation-induced cell death by enhancing FASL expression and suppressing cFLIP expression[168,169]. In addition to these active mechanisms of T cell death induction, there remains substantial evidence that the absence of survival signals mediated by cytokines contributes to T cell death, termed 'cytokine withdrawal-induced cell death' or 'activated cell autonomous death'. In this case, the absence of cytokine signalling reduces the levels of the antiapoptotic factors BCL-2 and BCL-$X_L$, leading to increased expression and activity of BIM, which ultimately results in T cell apoptosis[158,161,170].

Apart from apoptosis, there are multiple other mechanisms of T cell death that may contribute to peripheral T cell tolerance. One example is necroptosis (also called 'programmed necrosis', a type of programmed cell death that is caspase-independent and kinase RIPK3-dependent), which has been observed in T cells that are deficient in caspase 8 on TNF receptor signalling, impyling that caspase 8 is an inhibitor of necroptosis[171]. Ferroptosis (a type of programmed cell death that is dependent on iron) is induced through the accumulation of ROS that lead to membrane lipid peroxidation and subsequent cell death. It was recently shown that the ROS scavenger GPX4 is a critical survival mediator expressed by peripheral effector and memory T cells but not by thymocytes[172]. More importantly, several studies demonstrated that T follicular helper cells ($T_{FH}$ cells) are uniquely susceptible to caspase-dependent pyroptosis (a type of pro-inflammatory lytic programmed cell death that is triggered by inflammasome activation and mediated by caspase 1 (or caspase 11)) by the ionotropic ATP-gated receptor $P2X_7$ in response to ATP[173,174]. Two very recent reports demonstrated pyroptosis in resting (unactivated) human T cells, where it was induced by the activation of caspase 1 through the inflammasome sensor CARD8. This is an exciting finding as this mechanism likely depends on danger signals and not TCR signalling[175,176]. This death pathway may therefore control the number of $T_{FH}$ cells and may restrain the pathogenic $T_{FH}$ cell states that are observed in conditions such as lupus erythematosus[173].

Despite the complex multilayered networks of death pathways in T cell biology, there is an appreciation that this tolerance checkpoint mechanism remains the most efficient and most reliable means to restrain T cell expansion at almost every stage of T cell activity. This enables the control of the magnitude and timing of the immune response and thereby prevents the onset of T cell-mediated immunopathology.

## Conclusion

The identification of numerous genes and networks that regulate T cell tolerance has yielded clues about the mechanisms that protect us from hyperinflammation and autoimmunity. Here, we argue that intrinsic T cell tolerance is regulated by multiple mechanisms that work in harmony to achieve both constitutive and negative-feedback regulatory mechanisms and sets a strong barrier against pathologic inflammation. Nevertheless, several outstanding questions remain about the relative contribution of these mechanisms to protection versus immune dysregulation. For example, it remains unclear how the constitutive regulators of naive T cell quiescence cooperate in maintaining this state, and the molecular activities of most of the factors involved remain elusive. Another ill-defined aspect is the impact of anergy versus death versus exhaustion in the induction of T cell tolerance in cancer. Given the lack of mouse studies on T cell senescence, the contribution of this mechanism to T cell tolerance in different settings (for example, viral infections) and its effector molecules remain to be better defined. Peripheral deletional tolerance has been appreciated as a tolerance regulatory mechanism for decades. Despite this, we remain ignorant of the potential contribution of cell death mechanisms such as necroptosis and ferroptosis to T cell fate and regulation. Finally, we have reached the stage where complex phenotyping of immune cells allows unprecedented resolution of cell states, including multiple T cell tolerance states in the same model system, with exquisite temporal resolution. This enables us to ask the question of what tolerance mechanisms predominate in each setting and what mediators are at play.

*Mohamed A. ElTanbouly and Randolph J. Noelle*

*Department of Microbiology and Immunology, Geisel School of Medicine, Norris Cotton Cancer Center, Dartmouth College, Hanover, NH, USA.*

✉*e-mail: randolph.j.noelle@dartmouth.edu*

https://doi.org/10.1038/s41577-020-00454-2

Published online 19 October 2020

1. Rouse, B. T. & Sehrawat, S. Immunity and immunopathology to viruses: what decides the outcome? *Nat. Rev. Immunol.* **10**, 514–526 (2010).
2. Skapenko, A., Leipe, J., Lipsky, P. E. & Schulze-Koops, H. The role of the T cell in autoimmune inflammation. *Arthritis Res. Ther.* **7**, S4–S14 (2005).
3. Bouneaud, C., Kourilsky, P. & Bousso, P. Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. *Immunity* **13**, 829–840 (2000).
4. Gallegos, A. M. & Bevan, M. J. Central tolerance: good but imperfect. *Immunol. Rev.* **209**, 290–296 (2006).
5. Liu, G. Y. et al. Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity* **3**, 407–415 (1995).
6. Sakaguchi, S., Yamaguchi, T., Nomura, T. & Ono, M. Regulatory T cells and immune tolerance. *Cell* **133**, 775–787 (2008).
7. Saligrama, N. et al. Opposing T cell responses in experimental autoimmune encephalomyelitis. *Nature* **572**, 481–487 (2019).
8. Hasegawa, H. & Matsumoto, T. Mechanisms of tolerance induction by dendritic cells in vivo. *Front. Immunol.* **9**, 350 (2018).
9. Zhang, S. et al. Newly generated CD4$^+$ T cells acquire metabolic quiescence after thymic egress. *J. Immunol.* **200**, 1064–1077 (2018).
10. Chapman, N. M., Boothby, M. R. & Chi, H. Metabolic coordination of T cell quiescence and activation. *Nat. Rev. Immunol.* **20**, 55–70 (2020).
11. Hamilton, S. E. & Jameson, S. C. CD8 T cell quiescence revisited. *Trends Immunol.* **33**, 224–230 (2012).
12. Wildey, G. M. & Howe, P. H. Runx1 is a co-activator with FOXO3 to mediate transforming growth factor beta (TGFbeta)-induced Bim transcription in hepatic cells. *J. Biol. Chem.* **284**, 20227–20239 (2009).
13. Tu, E. et al. T cell receptor-regulated TGF-beta type I receptor expression determines T cell quiescence and activation. *Immunity* **48**, 745–759 (2018).
14. Tzachanis, D. & Boussiotis, V. A. Tob, a member of the APRO family, regulates immunological quiescence and tumor suppression. *Cell Cycle* **8**, 1019–1025 (2009).
15. Tzachanis, D. et al. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. *Nat. Immunol.* **2**, 1174–1182 (2001).
16. Matsuda, S., Rouault, J., Magaud, J. & Berthet, C. In search of a function for the TIS21/PC3/BTG1/TOB family. *FEBS Lett.* **497**, 67–72 (2001).
17. ElTanbouly, M. A. et al. VISTA is a checkpoint regulator for naive T cell quiescence and peripheral tolerance. *Science* https://doi.org/10.1126/science.aay0524 (2020).

18. Hwang, S. S. et al. mRNA destabilization by BTG1 and BTG2 maintains T cell quiescence. *Science* **367**, 1255–1260 (2020).
19. Howden, A. J. M. et al. Quantitative analysis of T cell proteomes and environmental sensors during T cell differentiation. *Nat. Immunol.* **20**, 1542–1554 (2019).
20. Buckley, A. F., Kuo, C. T. & Leiden, J. M. Transcription factor LKLF is sufficient to program T cell quiescence via a c-Myc-dependent pathway. *Nat. Immunol.* **2**, 698–704 (2001).
21. Haaland, R. E., Yu, W. & Rice, A. P. Identification of LKLF-regulated genes in quiescent CD4+ T lymphocytes. *Mol. Immunol.* **42**, 627–641 (2005).
22. Kuo, C. T., Veselits, M. L. & Leiden, J. M. LKLF: A transcriptional regulator of single-positive T cell quiescence and survival. *Science* **277**, 1986–1990 (1997).
23. Wu, J. & Lingrel, J. B. KLF2 inhibits Jurkat T leukemia cell growth via upregulation of cyclin-dependent kinase inhibitor p21WAF1/CIP1. *Oncogene* **23**, 8088–8096 (2004).
24. Bai, A., Hu, H., Yeung, M. & Chen, J. Kruppel-like factor 2 controls T cell trafficking by activating L-selectin (CD62L) and sphingosine-1-phosphate receptor 1 transcription. *J. Immunol.* **178**, 7632–7639 (2007).
25. Carlson, C. M. et al. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* **442**, 299–302 (2006).
26. Kerdiles, Y. M. et al. Foxo1 links homing and survival of naive T cells by regulating L-selectin, CCR7 and interleukin 7 receptor. *Nat. Immunol.* **10**, 176–184 (2009).
27. Ouyang, W., Beckett, O., Flavell, R. A. & Li, M. O. An essential role of the Forkhead-box transcription factor Foxo1 in control of T cell homeostasis and tolerance. *Immunity* **30**, 358–371 (2009).
28. Ouyang, W. & Li, M. O. Foxo: in command of T lymphocyte homeostasis and tolerance. *Trends Immunol.* **32**, 26–33 (2011).
29. Wong, W. F. et al. The artificial loss of Runx1 reduces the expression of quiescence-associated transcription factors in CD4$^+$ T lymphocytes. *Mol. Immunol.* **68**, 223–233 (2015).
30. Wong, W. F. et al. Runx1 deficiency in CD4+ T cells causes fatal autoimmune inflammatory lung disease due to spontaneous hyperactivation of cells. *J. Immunol.* **188**, 5408–5420 (2012).
31. Wu, Q. et al. The tuberous sclerosis complex-mammalian target of rapamycin pathway maintains the quiescence and survival of naive T cells. *J. Immunol.* **187**, 1106–1112 (2011).
32. Yang, K., Neale, G., Green, D. R., He, W. & Chi, H. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. *Nat. Immunol.* **12**, 888–897 (2011).
33. Neama, A. F., Looi, C. Y. & Wong, W. F. in *Lymphocyte Updates - Cancer, Autoimmunity and Infection* (ed Isvoranu, G.) (InTechOpen, 2017).
34. Chang, M. et al. The ubiquitin ligase Peli1 negatively regulates T cell activation and prevents autoimmunity. *Nat. Immunol.* **12**, 1002–1009 (2011).
35. Gorelik, L. & Flavell, R. A. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* **12**, 171–181 (2000).
36. Rubtsov, Y. P. & Rudensky, A. Y. TGFbeta signalling in control of T-cell-mediated self-reactivity. *Nat. Rev. Immunol.* **7**, 443–453 (2007).
37. ElTanbouly, M. A., Schaafsma, E., Noelle, R. J. & Lines, J. L. VISTA: coming of age as a multi-lineage immune checkpoint. *Clin. Exp. Immunol.* https://doi.org/10.1111/cei.13415 (2020).
38. ElTanbouly, M. A., Croteau, W., Noelle, R. J. & Lines, J. L. VISTA: a novel immunotherapy target for normalizing innate and adaptive immunity. *Semin. Immunol.* **42**, 101308 (2019).
39. ElTanbouly, M. A. et al. VISTA is a checkpoint regulator for naïve T cell quiescence and peripheral tolerance. *Science* **367**, eaay0524 (2020).
40. Wang, L. et al. Disruption of the immune-checkpoint VISTA gene imparts a proinflammatory phenotype with predisposition to the development of autoimmunity. *Proc. Natl Acad. Sci. USA* **111**, 14846–14851 (2014).
41. van den Broek, T., Borghans, J. A. M. & van Wijk, F. The full spectrum of human naive T cells. *Nat. Rev. Immunol.* **18**, 363–373 (2018).
42. Baranzini, S. E. The role of antiproliferative gene Tob1 in the immune system. *Clin. Exp. Neuroimmunol.* **5**, 132–136 (2014).
43. Bista, P., Mele, D. A., Baez, D. V. & Huber, B. T. Lymphocyte quiescence factor Dpp2 is transcriptionally activated by KLF2 and TOB1. *Mol. Immunol.* **45**, 3618–3623 (2008).
44. Wolf, T. et al. Dynamics in protein translation sustaining T cell preparedness. *Nat. Immunol.* https://doi.org/10.1038/s41590-020-0714-5 (2020).
45. Parish, I. A. & Heath, W. R. Too dangerous to ignore: self-tolerance and the control of ignorant autoreactive T cells. *Immunol. Cell Biol.* **86**, 146–152 (2008).
46. Janeway C. A. Jr., Travers, P., Walport, M. & Shlomchik, M. J. *Immunobiology. the immune system in health and disease* 5th edn (Garland Science, 2001).
47. Legoux, F. P. et al. CD4+ T cell tolerance to tissue-restricted self antigens is mediated by antigen-specific regulatory T cells rather than deletion. *Immunity* **43**, 896–908 (2015).
48. Malhotra, D. et al. Tolerance is established in polyclonal CD4$^+$ T cells by distinct mechanisms, according to self-peptide expression patterns. *Nat. Immunol.* **17**, 187–195 (2016).
49. Kurts, C. et al. CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc. Natl Acad. Sci. USA* **96**, 12703–12707 (1999).
50. Ohashi, P. S. et al. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell* **65**, 305–317 (1991).
51. Oldstone, M. B., Nerenberg, M., Southern, P., Price, J. & Lewicki, H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell* **65**, 319–331 (1991).
52. Cao, Y. et al. Functional inflammatory profiles distinguish myelin-reactive T cells from patients with multiple sclerosis. *Sci. Transl Med.* **7**, 287ra274 (2015).
53. Danke, N. A., Koelle, D. M., Yee, C., Beheray, S. & Kwok, W. W. Autoreactive T cells in healthy individuals. *J. Immunol.* **172**, 5967–5972 (2004).
54. Snir, O. et al. Identification and functional characterization of T cells reactive to citrullinated vimentin in HLA-DRB1*0401-positive humanized mice and rheumatoid arthritis patients. *Arthritis Rheum.* **63**, 2873–2883 (2011).
55. Heath, W. R. et al. Autoimmune diabetes as a consequence of locally produced interleukin-2. *Nature* **359**, 547–549 (1992).
56. Ramanathan, S. et al. Exposure to IL-15 and IL-21 enables autoreactive CD8 T cells to respond to weak antigens and cause disease in a mouse model of autoimmune diabetes. *J. Immunol.* **186**, 5131–5141 (2011).
57. Miller, S. D. et al. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat. Med.* **3**, 1133–1136 (1997).
58. Vezys, V. & Lefrancois, L. Cutting edge: inflammatory signals drive organ-specific autoimmunity to normally cross-tolerizing endogenous antigen. *J. Immunol.* **169**, 6677–6680 (2002).
59. DeSilva, D. R., Feeser, W. S., Tancula, E. J. & Scherle, P. A. Anergic T cells are defective in both jun NH2-terminal kinase and mitogen-activated protein kinase signaling pathways. *J. Exp. Med.* **183**, 2017–2023 (1996).
60. Fields, P. E., Gajewski, T. F. & Fitch, F. W. Blocked Ras activation in anergic CD4+ T cells. *Science* **271**, 1276–1278 (1996).
61. Li, W., Whaley, C. D., Mondino, A. & Mueller, D. L. Blocked signal transduction to the ERK and JNK protein kinases in anergic CD4+ T cells. *Science* **271**, 1272–1276 (1996).
62. Zha, Y. et al. T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase-alpha. *Nat. Immunol.* **7**, 1166–1173 (2006).
63. Kang, S. M. et al. Transactivation by AP-1 is a molecular target of T cell clonal anergy. *Science* **257**, 1134–1138 (1992).
64. Macian, F. et al. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell* **109**, 719–731 (2002).
65. Olenchock, B. A. et al. Disruption of diacylglycerol metabolism impairs the induction of T cell anergy. *Nat. Immunol.* **7**, 1174–1181 (2006).
66. Harris, J. E. et al. Early growth response gene-2, a zinc-finger transcription factor, is required for full induction of clonal anergy in CD4+ T cells. *J. Immunol.* **173**, 7331–7338 (2004).
67. Safford, M. et al. Egr-2 and Egr-3 are negative regulators of T cell activation. *Nat. Immunol.* **6**, 472–480 (2005).
68. Zheng, Y., Zha, Y., Driessens, G., Locke, F. & Gajewski, T. F. Transcriptional regulator early growth response gene 2 (Egr2) is required for T cell anergy in vitro and in vivo. *J. Exp. Med.* **209**, 2157–2163 (2012).
69. Bandyopadhyay, S. et al. Interleukin 2 gene transcription is regulated by Ikaros-induced changes in histone acetylation in anergic T cells. *Blood* **109**, 2878–2886 (2007).
70. Thomas, R. M., Saouaf, S. J. & Wells, A. D. Superantigen-induced CD4+ T cell tolerance is associated with DNA methylation and histone hypo-acetylation at cytokine gene loci. *Genes. Immun.* **8**, 613–618 (2007).
71. Villarino, A. V. et al. Posttranscriptional silencing of effector cytokine mRNA underlies the anergic phenotype of self-reactive T cells. *Immunity* **34**, 50–60 (2011).
72. Liu, X. et al. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature* **567**, 525–529 (2019).
73. Pape, K. A., Merica, R., Mondino, A., Khoruts, A. & Jenkins, M. K. Direct evidence that functionally impaired CD4+ T cells persist in vivo following induction of peripheral tolerance. *J. Immunol.* **160**, 4719–4729 (1998).
74. Rocha, B., Grandien, A. & Freitas, A. A. Anergy and exhaustion are independent mechanisms of peripheral T cell tolerance. *J. Exp. Med.* **181**, 993–1003 (1995).
75. Rocha, B., Tanchot, C. & Von Boehmer, H. Clonal anergy blocks in vivo growth of mature T cells and can be reversed in the absence of antigen. *J. Exp. Med.* **177**, 1517–1521 (1993).
76. Brown, I. E., Blank, C., Kline, J., Kacha, A. K. & Gajewski, T. F. Homeostatic proliferation as an isolated variable reverses CD8+ T cell anergy and promotes tumor rejection. *J. Immunol.* **177**, 4521–4529 (2006).
77. Martinez, R. J. et al. Arthritogenic self-reactive CD4+ T cells acquire an FR4hiCD73hi anergic state in the presence of Foxp3+ regulatory T cells. *J. Immunol.* **188**, 170–181 (2012).
78. Kalekar, L. A. et al. CD4$^+$ T cell anergy prevents autoimmunity and generates regulatory T cell precursors. *Nat. Immunol.* **17**, 304–314 (2016).
79. Kalekar, L. A. & Mueller, D. L. Relationship between CD4 regulatory T cells and anergy in vivo. *J. Immunol.* **198**, 2527–2533 (2017).
80. Wells, A. D. New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting. *J. Immunol.* **182**, 7331–7341 (2009).
81. Delgoffe, G. M. & Powell, J. D. Feeding an army: the metabolism of T cells in activation, anergy, and exhaustion. *Mol. Immunol.* **68**, 492–496 (2015).
82. Zheng, Y., Delgoffe, G. M., Meyer, C. F., Chan, W. & Powell, J. D. Anergic T cells are metabolically anergic. *J. Immunol.* **183**, 6095–6101 (2009).
83. Powell, J. D. & Delgoffe, G. M. The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. *Immunity* **33**, 301–311 (2010).
84. Li, L., Iwamoto, Y., Berezovskaya, A. & Boussiotis, V. A. A pathway regulated by cell cycle inhibitor p27Kip1 and checkpoint inhibitor Smad3 is involved in the induction of T cell tolerance. *Nat. Immunol.* **7**, 1157–1165 (2006).
85. Williams, J. B. et al. The EGR2 targets LAG-3 and 4-1BB describe and regulate dysfunctional antigen-specific CD8+ T cells in the tumor microenvironment. *J. Exp. Med.* **214**, 381–400 (2017).
86. Maeda, Y. et al. Detection of self-reactive CD8$^+$ T cells with an anergic phenotype in healthy individuals. *Science* **346**, 1536–1540 (2014).
87. Gallimore, A. et al. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med.* **187**, 1383–1393 (1998).
88. Zajac, A. J. et al. Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188**, 2205–2213 (1998).
89. Fuller, M. J. et al. Cutting edge: emergence of CD127high functionally competent memory T cells is compromised by high viral loads and inadequate T cell help. *J. Immunol.* **174**, 5926–5930 (2005).
90. Wherry, E. J. T cell exhaustion. *Nat. Immunol.* **12**, 492–499 (2011).
91. Wherry, E. J., Barber, D. L., Kaech, S. M., Blattman, J. N. & Ahmed, R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc. Natl Acad. Sci. USA* **101**, 16004–16009 (2004).
92. Blackburn, S. D. et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat. Immunol.* **10**, 29–37 (2009).
93. Wherry, E. J. et al. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* **27**, 670–684 (2007).

94. Fuertes Marraco, S. A., Neubert, N. J., Verdeil, G. & Speiser, D. E. Inhibitory receptors beyond T cell exhaustion. *Front. Immunol.* **6**, 310 (2015).
95. Legat, A., Speiser, D. E., Pircher, H., Zehn, D. & Fuertes Marraco, S. A. Inhibitory receptor expression depends more dominantly on differentiation and activation than "exhaustion" of human CD8 T cells. *Front. Immunol.* **4**, 455 (2013).
96. Utzschneider, D. T. et al. T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. *Nat. Immunol.* **14**, 603–610 (2013).
97. Baitsch, L. et al. Extended co-expression of inhibitory receptors by human CD8 T-cells depending on differentiation, antigen-specificity and anatomical localization. *PLoS ONE* **7**, e30852 (2012).
98. Duraiswamy, J. et al. Phenotype, function, and gene expression profiles of programmed death-1$^{hi}$ CD8 T cells in healthy human adults. *J. Immunol.* **186**, 4200–4212 (2011).
99. Angelosanto, J. M., Blackburn, S. D., Crawford, A. & Wherry, E. J. Progressive loss of memory T cell potential and commitment to exhaustion during chronic viral infection. *J. Virol.* **86**, 8161–8170 (2012).
100. Blank, C. U. et al. Defining 'T cell exhaustion'. *Nat. Rev. Immunol.* **19**, 665–674 (2019).
101. Collins, M. H. & Henderson, A. J. Transcriptional regulation and T cell exhaustion. *Curr. Opin. HIV. AIDS* **9**, 459–463 (2014).
102. Quigley, M. et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat. Med.* **16**, 1147–1151 (2010).
103. Seo, H. et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8$^+$ T cell exhaustion. *Proc. Natl Acad. Sci. USA* **116**, 12410–12415 (2019).
104. Alfei, F. et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* **571**, 265–269 (2019).
105. Khan, O. et al. TOX transcriptionally and epigenetically programs CD8$^+$ T cell exhaustion. *Nature* https://doi.org/10.1038/s41586-019-1325-x (2019).
106. Scott, A. C. et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* https://doi.org/10.1038/s41586-019-1324-y (2019).
107. Kallies, A., Zehn, D. & Utzschneider, D. T. Precursor exhausted T cells: key to successful immunotherapy? *Nat. Rev. Immunol.* **20**, 128–136 (2020).
108. Im, S. J. et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **537**, 417–421 (2016).
109. Utzschneider, D. T. et al. T cell factor 1-expressing memory-like CD8$^+$ T cells sustain the immune response to chronic viral infections. *Immunity* **45**, 415–427 (2016).
110. Kratchmarov, R., Magun, A. M. & Reiner, S. L. TCF1 expression marks self-renewing human CD8$^+$ T cells. *Blood Adv.* **2**, 1685–1690 (2018).
111. McKinney, E. F., Lee, J. C., Jayne, D. R., Lyons, P. A. & Smith, K. G. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* **523**, 612–616 (2015).
112. Zielinski, M. et al. Impact of donor and recipient human cytomegalovirus status on kidney transplantation. *Int. Immunol.* **29**, 541–549 (2017).
113. Shahbazi, M., Soltanzadeh-Yamchi, M. & Mohammadnia-Afrouzi, M. T cell exhaustion implications during transplantation. *Immunol. Lett.* **202**, 52–58 (2018).
114. Steger, U. et al. Exhaustive differentiation of alloreactive CD8+ T cells: critical for determination of graft acceptance or rejection. *Transplantation* **85**, 1339–1347 (2008).
115. Wang, H. et al. Prevention of allograft rejection in heart transplantation through concurrent gene silencing of TLR and kinase signaling pathways. *Sci. Rep.* **6**, 33869 (2016).
116. Frebel, H. et al. Programmed death 1 protects from fatal circulatory failure during systemic virus infection of mice. *J. Exp. Med.* **209**, 2485–2499 (2012).
117. Zinselmeyer, B. H. et al. PD-1 promotes immune exhaustion by inducing antiviral T cell motility paralysis. *J. Exp. Med.* **210**, 757–774 (2013).
118. Gil, J. Cellular senescence causes ageing. *Nat. Rev. Mol. Cell Biol.* **20**, 388 (2019).
119. Reed, J. R. et al. Telomere erosion in memory T cells induced by telomerase inhibition at the site of antigenic challenge in vivo. *J. Exp. Med.* **199**, 1433–1443 (2004).
120. Hernandez-Segura, A., Nehme, J. & Demaria, M. Hallmarks of cellular senescence. *Trends Cell Biol.* **28**, 436–453 (2018).
121. Hildeman, D. A., Mitchell, T., Kappler, J. & Marrack, P. T cell apoptosis and reactive oxygen species. *J. Clin. Invest.* **111**, 575–581 (2003).
122. Hildeman, D. A. et al. Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity* **10**, 735–744 (1999).
123. Yarosz, E. L. & Chang, C. H. The role of reactive oxygen species in regulating T cell-mediated immunity and disease. *Immune Netw.* **18**, e14 (2018).
124. Akbar, A. N., Henson, S. M. & Lanna, A. Senescence of T lymphocytes: implications for enhancing human immunity. *Trends Immunol.* **37**, 866–876 (2016).
125. Libri, V. et al. Cytomegalovirus infection induces the accumulation of short-lived, multifunctional CD4+CD45RA+CD27+ T cells: the potential involvement of interleukin-7 in this process. *Immunology* **132**, 326–339 (2011).
126. Plunkett, F. J. et al. The loss of telomerase activity in highly differentiated CD8+CD28-CD27- T cells is associated with decreased Akt (Ser473) phosphorylation. *J. Immunol.* **178**, 7710–7719 (2007).
127. Henson, S. M., Macaulay, R., Riddell, N. E., Nunn, C. J. & Akbar, A. N. Blockade of PD-1 or p38 MAP kinase signaling enhances senescent human CD8$^+$ T-cell proliferation by distinct pathways. *Eur. J. Immunol.* **45**, 1441–1451 (2015).
128. Di Mitri, D. et al. Reversible senescence in human CD4+CD45RA+CD27- memory T cells. *J. Immunol.* **187**, 2093–2100 (2011).
129. Lanna, A., Henson, S. M., Escors, D. & Akbar, A. N. The kinase p38 activated by the metabolic regulator AMPK and scaffold TAB1 drives the senescence of human T cells. *Nat. Immunol.* **15**, 965–972 (2014).
130. Chou, J. P. & Effros, R. B. T cell replicative senescence in human aging. *Curr. Pharm. Des.* **19**, 1680–1698 (2013).
131. Goronzy, J. J. & Weyand, C. M. Aging, autoimmunity and arthritis: T-cell senescence and contraction of T-cell repertoire diversity - catalysts of autoimmunity and chronic inflammation. *Arthritis Res. Ther.* **5**, 225–234 (2003).
132. Zhao, Y., Shao, Q. & Peng, G. Exhaustion and senescence: two crucial dysfunctional states of T cells in the tumor microenvironment. *Cell Mol. Immunol.* **17**, 27–35 (2020).
133. Pereira, B. I. et al. Sestrins induce natural killer function in senescent-like CD8$^+$ T cells. *Nat. Immunol.* https://doi.org/10.1038/s41590-020-0643-3 (2020).
134. Akbar, A. N., Beverley, P. C. & Salmon, M. Will telomere erosion lead to a loss of T-cell memory? *Nat. Rev. Immunol.* **4**, 737–743 (2004).
135. Aspinall, R., Del Giudice, G., Effros, R. B., Grubeck-Loebenstein, B. & Sambhara, S. Challenges for vaccination in the elderly. *Immun. Ageing* **4**, 9 (2007).
136. Grubeck-Loebenstein, B. et al. Immunosenescence and vaccine failure in the elderly. *Aging Clin. Exp. Res.* **21**, 201–209 (2009).
137. Beltran-Sanchez, H., Soneji, S. & Crimmins, E. M. Past, present, and future of healthy life expectancy. *Cold Spring Harb. Perspect. Med.* https://doi.org/10.1101/cshperspect.a025957 (2015).
138. Yoshikawa, T. T. Perspective: aging and infectious diseases: past, present, and future. *J. Infect. Dis.* **176**, 1053–1057 (1997).
139. Berger, R., Florent, G. & Just, M. Decrease of the lymphoproliferative response to varicella-zoster virus antigen in the aged. *Infect. Immun.* **32**, 24–27 (1981).
140. Fulop, T., Larbi, A. & Pawelec, G. Human T cell aging and the impact of persistent viral infections. *Front. Immunol.* **4**, 271 (2013).
141. Ouyang, Q. et al. An age-related increase in the number of CD8+ T cells carrying receptors for an immunodominant Epstein-Barr virus (EBV) epitope is counteracted by a decreased frequency of their antigen-specific responsiveness. *Mech. Ageing Dev.* **124**, 477–485 (2003).
142. Kurts, C., Kosaka, H., Carbone, F. R., Miller, J. F. & Heath, W. R. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8$^+$ T cells. *J. Exp. Med.* **186**, 239–245 (1997).
143. Kyburz, D. et al. T cell immunity after a viral infection versus T cell tolerance induced by soluble viral peptides. *Eur. J. Immunol.* **23**, 1956–1962 (1993).
144. Liblau, R. S. et al. Intravenous injection of soluble antigen induces thymic and peripheral T-cells apoptosis. *Proc. Natl Acad. Sci. USA* **93**, 3031–3036 (1996).
145. Davey, G. M. et al. Peripheral deletion of autoreactive CD8 T cells by cross presentation of self-antigen occurs by a Bcl-2-inhibitable pathway mediated by Bim. *J. Exp. Med.* **196**, 947–955 (2002).
146. Hildeman, D. A., Zhu, Y., Mitchell, T. C., Kappler, J. & Marrack, P. Molecular mechanisms of activated T cell death in vivo. *Curr. Opin. Immunol.* **14**, 354–359 (2002).
147. Boise, L. H. et al. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* **3**, 87–98 (1995).
148. Burr, J. S. et al. Cutting edge: distinct motifs within CD28 regulate T cell proliferation and induction of Bcl-XL. *J. Immunol.* **166**, 5331–5335 (2001).
149. Okkenhaug, K. et al. A point mutation in CD28 distinguishes proliferative signals from survival signals. *Nat. Immunol.* **2**, 325–332 (2001).
150. Rathmell, J. C., Farkash, E. A., Gao, W. & Thompson, C. B. IL-7 enhances the survival and maintains the size of naive T cells. *J. Immunol.* **167**, 6869–6876 (2001).
151. Silva-Filho, J. L., Caruso-Neves, C. & Pinheiro, A. A. S. IL-4: an important cytokine in determining the fate of T cells. *Biophys. Rev.* **6**, 111–118 (2014).
152. Parish, I. A. et al. The molecular signature of CD8+ T cells undergoing deletional tolerance. *Blood* **113**, 4575–4585 (2009).
153. Hochweller, K. & Anderton, S. M. Kinetics of costimulatory molecule expression by T cells and dendritic cells during the induction of tolerance versus immunity in vivo. *Eur. J. Immunol.* **35**, 1086–1096 (2005).
154. Rajpal, A. et al. Transcriptional activation of known and novel apoptotic pathways by Nur77 orphan steroid receptor. *EMBO J.* **22**, 6526–6536 (2003).
155. Barron, L., Knoechel, B., Lohr, J. & Abbas, A. K. Cutting edge: contributions of apoptosis and anergy to systemic T cell tolerance. *J. Immunol.* **180**, 2762–2766 (2008).
156. Green, D. R., Droin, N. & Pinkoski, M. Activation-induced cell death in T cells. *Immunol. Rev.* **193**, 70–81 (2003).
157. Kawabe, Y. & Ochi, A. Programmed cell death and extrathymic reduction of Vbeta8+ CD4+ T cells in mice tolerant to Staphylococcus aureus enterotoxin B. *Nature* **349**, 245–248 (1991).
158. Snow, A. L., Pandiyan, P., Zheng, L., Krummey, S. M. & Lenardo, M. J. The power and the promise of restimulation-induced cell death in human immune diseases. *Immunol. Rev.* **236**, 68–82 (2010).
159. Dhein, J., Walczak, H., Baumler, C., Debatin, K. M. & Krammer, P. H. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* **373**, 438–441 (1995).
160. Krammer, P. H. CD95's deadly mission in the immune system. *Nature* **407**, 789–795 (2000).
161. Krammer, P. H., Arnold, R. & Lavrik, I. N. Life and death in peripheral T cells. *Nat. Rev. Immunol.* **7**, 532–542 (2007).
162. Suzuki, I. & Fink, P. J. The dual functions of fas ligand in the regulation of peripheral CD8+ and CD4+ T cells. *Proc. Natl Acad. Sci. USA* **97**, 1707–1712 (2000).
163. Tham, E. L. & Mescher, M. F. The poststimulation program of CD4 versus CD8 T cells (death versus activation-induced nonresponsiveness). *J. Immunol.* **169**, 1822–1828 (2002).
164. Zheng, L. et al. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* **377**, 348–351 (1995).
165. Kirchhoff, S., Muller, W. W., Krueger, A., Schmitz, I. & Krammer, P. H. TCR-mediated up-regulation of c-FLIPshort correlates with resistance toward CD95-mediated apoptosis by blocking death-inducing signaling complex activity. *J. Immunol.* **165**, 6293–6300 (2000).
166. Kaech, S. M. et al. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol.* **4**, 1191–1198 (2003).
167. Kalia, V. et al. Prolonged interleukin-2Ralpha expression on virus-specific CD8+ T cells favors terminal-effector differentiation in vivo. *Immunity* **32**, 91–103 (2010).
168. Lenardo, M. J. Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. *Nature* **353**, 858–861 (1991).
169. Refaeli, Y., Parijs, L. V., London, C. A., Tschopp, J. & Abbas, A. K. Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity* **8**, 615–623 (1998).
170. Marrack, P. & Kappler, J. Control of T cell viability. *Annu. Rev. Immunol.* **22**, 765–787 (2004).

171. Ch'en, I. L., Tsau, J. S., Molkentin, J. D., Komatsu, M. & Hedrick, S. M. Mechanisms of necroptosis in T cells. *J. Exp. Med.* **208**, 633–641 (2011).
172. Matsushita, M. et al. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. *J. Exp. Med.* **212**, 555–568 (2015).
173. Faliti, C. E. et al. P2X7 receptor restrains pathogenic Tfh cell generation in systemic lupus erythematosus. *J. Exp. Med.* **216**, 317–336 (2019).
174. Kunzli, M. et al. Long-lived T follicular helper cells retain plasticity and help sustain humoral immunity. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.aay5552 (2020).
175. Andreas Linder, S. B. et al. CARD8 inflammasome activation triggers pyroptosis in human T cells. *EMBO J.* https://doi.org/10.15252/embj.2020105071 (2020).
176. Johnson, D. C. et al. DPP8/9 inhibitors activate the CARD8 inflammasome in resting lymphocytes. *Cell Death Dis.* **11**, 628 (2020).
177. Barber, D. L. et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* **439**, 682–687 (2006).
178. Johnston, R. J. et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8$^+$ T cell effector function. *Cancer Cell* **26**, 923–937 (2014).
179. Yang, Z. Z. et al. Expression of LAG-3 defines exhaustion of intratumoral PD-1$^+$ T cells and correlates with poor outcome in follicular lymphoma. *Oncotarget* **8**, 61425–61439 (2017).
180. Sakuishi, K. et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J. Exp. Med.* **207**, 2187–2194 (2010).
181. Otano, I. et al. Human CD8 T cells are susceptible to TNF-mediated activation-induced cell death. *Theranostics* **10**, 4481–4489 (2020).
182. Janssen, E. M. et al. CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* **434**, 88–93 (2005).
183. Martinez-Lorenzo, M. J. et al. Involvement of APO2 ligand/TRAIL in activation-induced death of Jurkat and human peripheral blood T cells. *Eur. J. Immunol.* **28**, 2714–2725 (1998).
184. Whiting, C. C., Su, L. L., Lin, J. T. & Fathman, C. G. GRAIL: a unique mediator of CD4 T-lymphocyte unresponsiveness. *FEBS J.* **278**, 47–58 (2011).
185. Fang, D. et al. Dysregulation of T lymphocyte function in itchy mice: a role for Itch in TH2 differentiation. *Nat. Immunol.* **3**, 281–287 (2002).
186. Venuprasad, K. Cbl-b and itch: key regulators of peripheral T-cell tolerance. *Cancer Res.* **70**, 3009–3012 (2010).
187. Chemnitz, J. M., Parry, R. V., Nichols, K. E., June, C. H. & Riley, J. L. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J. Immunol.* **173**, 945–954 (2004).
188. Yokosuka, T. et al. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J. Exp. Med.* **209**, 1201–1217 (2012).
189. LaFleur, M. W. et al. PTPN2 regulates the generation of exhausted CD8$^+$ T cell subpopulations and restrains tumor immunity. *Nat. Immunol.* **20**, 1335–1347 (2019).
190. Masson, F., Kupresanin, F., Mount, A., Strasser, A. & Belz, G. T. Bid and Bim collaborate during induction of T cell death in persistent infection. *J. Immunol.* **186**, 4059–4066 (2011).
191. Garaud, S. et al. FOXP1 is a regulator of quiescence in healthy human CD4$^+$ T cells and is constitutively repressed in T cells from patients with lymphoproliferative disorders. *Eur. J. Immunol.* **47**, 168–179 (2017).
192. Wei, H. et al. Cutting edge: Foxp1 controls naive CD8+ T cell quiescence by simultaneously repressing key pathways in cellular metabolism and cell cycle progression. *J. Immunol.* **196**, 3537–3541 (2016).
193. Man, K. et al. Transcription factor IRF4 promotes CD8$^+$ T cell exhaustion and limits the development of memory-like T cells during chronic infection. *Immunity* **47**, 1129–1141 (2017).
194. Singer, M. et al. A distinct gene module for dysfunction uncoupled from activation in tumor-infiltrating T cells. *Cell* **166**, 1500–1511 (2016).
195. Shin, H. et al. A role for the transcriptional repressor Blimp-1 in CD8+ T cell exhaustion during chronic viral infection. *Immunity* **31**, 309–320 (2009).
196. Li, J., He, Y., Hao, J., Ni, L. & Dong, C. High levels of Eomes promote exhaustion of anti-tumor CD8$^+$ T cells. *Front. Immunol.* **9**, 2981 (2018).
197. Sprent, J. & Surh, C. D. Normal T cell homeostasis: the conversion of naive cells into memory-phenotype cells. *Nat. Immunol.* **12**, 478–484 (2011).
198. Allam, A. et al. The CD8+ memory T-cell state of readiness is actively maintained and reversible. *Blood* **114**, 2121–2130 (2009).
199. Latner, D. R., Kaech, S. M. & Ahmed, R. Enhanced expression of cell cycle regulatory genes in virus-specific memory CD8+ T cells. *J. Virol.* **78**, 10953–10959 (2004).
200. Youngblood, B. et al. Effector CD8 T cells dedifferentiate into long-lived memory cells. *Nature* **552**, 404–409 (2017).
201. Gebhardt, T. et al. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat. Immunol.* **10**, 524–530 (2009).
202. Milner, J. J. et al. Heterogenous populations of tissue-resident CD8$^+$ T cells are generated in response to infection and malignancy. *Immunity* **52**, 808–824 (2020).
203. Carbone, F. R. Immovable memories: the journey to permanent residency. *Nat. Immunol.* **21**, 698–699 (2020).
204. Driessens, G., Kline, J. & Gajewski, T. F. Costimulatory and coinhibitory receptors in anti-tumor immunity. *Immunol. Rev.* **229**, 126–144 (2009).
205. Tirapu, I. et al. Low surface expression of B7-1 (CD80) is an immunoescape mechanism of colon carcinoma. *Cancer Res.* **66**, 2442–2450 (2006).
206. Wu, T. C., Huang, A. Y., Jaffee, E. M., Levitsky, H. I. & Pardoll, D. M. A reassessment of the role of B7-1 expression in tumor rejection. *J. Exp. Med.* **182**, 1415–1421 (1995).
207. Zou, W. & Chen, L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat. Rev. Immunol.* **8**, 467–477 (2008).
208. Cuenca, A. et al. Extra-lymphatic solid tumor growth is not immunologically ignored and results in early induction of antigen-specific T-cell anergy: dominant role of cross-tolerance to tumor antigens. *Cancer Res.* **63**, 9007–9015 (2003).
209. Mescher, M. F., Popescu, F. E., Gerner, M., Hammerbeck, C. D. & Curtsinger, J. M. Activation-induced non-responsiveness (anergy) limits CD8 T cell responses to tumors. *Semin. Cancer Biol.* **17**, 299–308 (2007).
210. Staveley-O'Carroll, K. et al. Induction of antigen-specific T cell anergy: an early event in the course of tumor progression. *Proc. Natl Acad. Sci. USA* **95**, 1178–1183 (1998).
211. Abe, B. T., Shin, D. S., Mocholi, E. & Macian, F. NFAT1 supports tumor-induced anergy of CD4$^+$ T cells. *Cancer Res.* **72**, 4642–4651 (2012).
212. Verma, V. et al. PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1$^+$CD38$^{hi}$ cells and anti-PD-1 resistance. *Nat. Immunol.* **20**, 1231–1243 (2019).
213. Greenwald, R. J., Boussiotis, V. A., Lorsbach, R. B., Abbas, A. K. & Sharpe, A. H. CTLA-4 regulates induction of anergy in vivo. *Immunity* **14**, 145–155 (2001).
214. Liu, Y. & Zheng, P. How does an anti-CTLA-4 antibody promote cancer immunity? *Trends Immunol.* **39**, 953–956 (2018).
215. Redmond, W. L., Gough, M. J. & Weinberg, A. D. Ligation of the OX40 co-stimulatory receptor reverses self-Ag and tumor-induced CD8 T-cell anergy in vivo. *Eur. J. Immunol.* **39**, 2184–2194 (2009).
216. Alonso, R. et al. Induction of anergic or regulatory tumor-specific CD4$^+$ T cells in the tumor-draining lymph node. *Nat. Commun.* **9**, 2113 (2018).
217. Magen, A. et al. Single-cell profiling defines transcriptomic signatures specific to tumor-reactive versus virus-responsive CD4$^+$ T cells. *Cell Rep.* **29**, 3019–3032 e3016 (2019).
218. Speiser, D. E., Ho, P. C. & Verdeil, G. Regulatory circuits of T cell function in cancer. *Nat. Rev. Immunol.* **16**, 599–611 (2016).
219. Kahan, S. M., Wherry, E. J. & Zajac, A. J. T cell exhaustion during persistent viral infections. *Virology* **479–480**, 180–193 (2015).
220. Schietinger, A. et al. Tumor-specific T cell dysfunction is a dynamic antigen-driven differentiation program initiated early during tumorigenesis. *Immunity* **45**, 389–401 (2016).
221. Willimsky, G. & Blankenstein, T. Sporadic immunogenic tumours avoid destruction by inducing T-cell tolerance. *Nature* **437**, 141–146 (2005).
222. Pauken, K. E. et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* **354**, 1160–1165 (2016).
223. Yost, K. E. et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat. Med.* **25**, 1251–1259 (2019).



**Acknowledgements**
The authors thank S. Hedrick and C. Burns for their valuable suggestions and discussion.


**Author contributions**
The authors contributed equally to all aspects of the article.

**Competing interests**
R.J.N. is an inventor on patent applications (10035857, 9631018, 9217035, 8501915, 8465740, 8236304 and 8231872) submitted by Dartmouth College, and patent applications (9890215 and 9381244) submitted by Kings College London and Dartmouth College and is a co-founder of ImmuNext, a company involved in the development of VISTA-related assets. These applications cover the use of VISTA targeting for modulation of the immune response. M.A.E. declares no competing interests.


**Peer review information**
*Nature Reviews Immunology* thanks Daniel Utzschneider, Vassiliki Boussiotis and Pamela Ohashi for their contribution to the peer review of this work.