The natural history of naive T cells from birth to maturity

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Summary

Generating and maintaining a diverse repertoire of naive T cells is essential for protection against pathogens, and developing a mechanistic and quantitative description of the processes involved lies at the heart of our understanding of vertebrate immunity. Here we review the biology of naive T cells from birth to maturity and outline how the integration of mathematical models and experiments has helped us to develop a fuller picture of their life-histories.

1 | Introduction

T cells are a key component of the adaptive immune system and have the capacity to specifically recognise pathogens and mount a sterilising immune response to the infection. The persistence of specific T cells after an immune response is the basis of immunological memory, and for many pathogens a single infectious encounter results in life-long immunity to further exposures. The diverse repertoire of antigen receptors expressed by T cells represents a potent weapon for the immune system but also introduces unique challenges. Generation and maintenance of the naive (antigen-inexperienced) T cell compartment is a complex, multi-staged process, that must balance repertoire diversity with self-tolerance. Consequently, there has been great interest in understanding the developmental processes responsible for generating and maintaining this repertoire. Much of this work has been qualitative, identifying the extrinsic signals and genetic programmes that direct different stages of T cell development. However almost all stages of a T cell's life history are fundamentally dynamic, arising from a series of balances between production, death or differentiation. Measuring the relative contributions of these processes and how each of them responds to physiological perturbations is important for understanding how vertebrates maintain large, diverse and tolerant T cell populations. Here we review examples of how quantitative approaches have contributed to a deeper understanding of naive T cell development and maintenance, from early stages in the thymus through to the periphery.

2 | Quantitative aspects of thymic development

Since the population biology of T lymphocytes as a whole involves very large numbers of cells, the majority of modelling approaches have employed ordinary differential equations (ODEs) to track the expected sizes of the population(s) of interest as they

undergo division, death or differentiation. The interpretation of the parameters in these models is then usually straightforward, although the expression for the expected time taken for cells to pass successfully through a sequence of maturation steps are perhaps unintuitive (Box 1). In what follows we highlight the methodology only in the few instances in which probabilistic, stochastic or agent-based models have been used.

2.1 | Thymic T cell precursors – lineage choices and expanding the repertoire

T cells develop from haematopoetic progenitors that reside in the bone marrow. Uncommitted lymphoid progenitors then migrate to the thymus as early thymic progenitors (ETP), where they can commit to a T lineage cell fate. Prior to T lineage commitment, ETP have the potential to develop into B cells, NK cells and dendritic cell lineages, and thymic NK and DC populations are physiologically recognised products of these early progenitors. The stages of T cell development in thymus are extremely well characterised[1,2] and illustrated in Figure 1. Briefly, expression of CD4 and CD8 co-receptors provides a low resolution overview of the process. Progenitors enter the thymus as double negative (DN) for both coreceptors, but upregulate both following successful rearrangement of *Tcr* genes and expression of a mature TCR to become CD4 CD8 double positive (DP). These DP cells then undergo a selection process that identifies cells with functional TCRs and then correlates onward lineage development into the CD4 or CD8 lineage with MHC restriction, resulting in down-regulation of CD4 in class I restricted cells to give CD8 lineage T cells, and the loss of CD8 expression by Class II restricted cells to give rise to CD4 lineage T cells.

The DN compartment includes early progenitor T cells that commit to the T cell lineage and start the process of *Trcb* gene arrangements that give rise to mature TCR structures. Expression of functional TCRβ chains stimulates cells to undergo extensive cell division and expansion, resulting in the generation of the large numbers of DPs required to audition for selection. These different stages of DN development can be conveniently identified on the basis of CD25 and CD44 expression. ETP enter the thymus and fall within the $CD44^+CD25^-$ (DN1) phenotype, further identified by expression of cKit[3]. Commitment to the T cell lineage occurs in $CD44^+CD25^+$ (DN2) cells, which can further be divided into cells that retain NK and dendritic cell potential[4,5] (DN2a) and those fully committed to T lineage (DN2b). *Trcb* gene rearrangement occurs in cells with a $CD44^-CD25^+$ (DN3) phenotype, which can also be further divided into those that have successfully rearranged their *Trcb* gene (DN3b) and those attempting to do so (DN3a). At this stage, it also possible for *Tcrd* and *Trcg* genes to be rearranged and divert development to a $\gamma\delta$ T cell lineage. Successful expression of a mature TCRβ in complex with the preTα receptor then drives a burst of cell division, and development through a $CD44^-CD25^-$ (DN4) phenotype. Division continues as DN4 cells upregulate CD8 and CD4 coreceptors to become DP cells, a stage we turn to in the next section.

While the DN compartment is one of the smallest in the thymus, it is the one in which cells spend the longest time, and various quantifications and models of progression through the DN stages are in general agreement regarding the timescales involved. Our own studies, tracking progeny of congenically marked haematopoietic stem cells in the steady state, suggest that it takes up to two weeks for the first donor cells to appear in DN2 and DN3 stages, and a further 2-3 weeks for complete turnover of the DN compartments[6], similar to observations of using experimental transfer of thymic progenitors[3]. Data from the latter study were used to model the DN1-2 transition[7], estimating transit times of around 10 days in DN1 and indicating that differentiation to DN2 occurs after multiple rounds of division, supported by the finding that *in vitro* differentiation of putative early

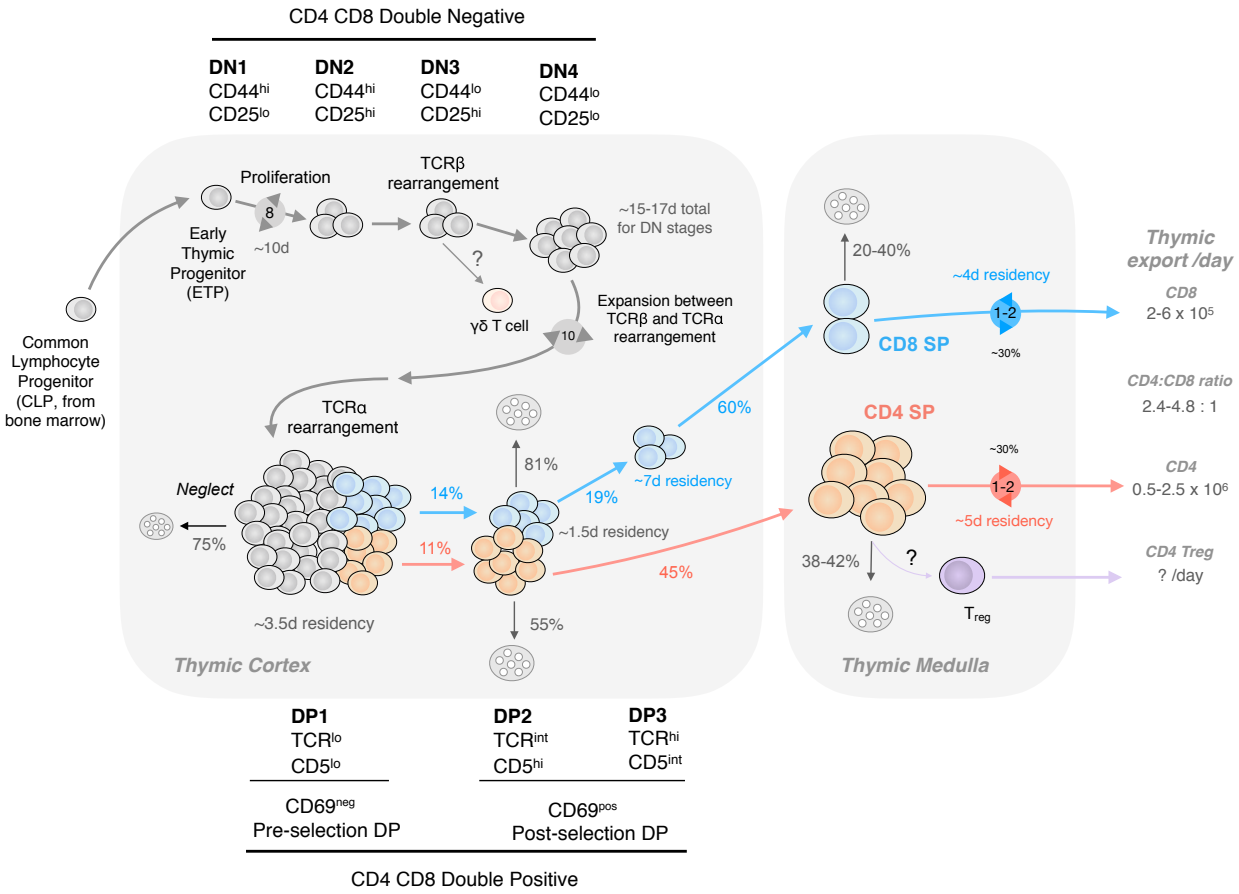

Figure 1: Mapping the development of $\alpha\beta$ T cells in the thymus. Common lymphoid progenitor cells migrate from the bone marrow to the thymus where they begin a multi-stage process of development. Red and blue denote the CD4+ and CD8+ lineages of TCR$\alpha\beta$ T cells respectively. Discs with arrows denote the approximate number of cell divisions undergone at each stage. DN - CD4 CD8 double negative, DP - CD4 CD8 double positive.

stage Flt3+ DN1 was inefficient and required more cell divisions than later stage Flt3$^-$ DN1 cells. Other quantitative studies have examined the DN compartment as a whole, modelling its reconstitution following depletion. Thomas-Vaslin *et al.*[8] fitted a multi-compartment ODE model of thymocyte development to data from drug induced ablation of cycling cells, which considered the four major CD4/CD8 developmental compartments (DN, DP, SP4, SP8). Souza-e-Silva et al[9] developed an agent-based model describing movement and behaviour of thymocytes in a 2D lattice following irradiation. These two very different approaches derived similar estimated average total DN residency times of 16-18d, in agreement with empirical observations[3]. Thomas-Vaslin *et al.*[8] estimated an average recruitment of 20,000 ETP per day, and that DN cells undergo an average of four cell divisions. While these parameters were sufficient to explain the size of the DN compartment size and its output, the granularity of the description may confound influx and proliferation. Studies enumerating ETP in the adult thymus reveal around 1,000 cells[10] and others suggest that entry of precursors into the thymus may be episodic rather than a steady flow[11]. A further level of complexity comes from studies suggesting that early thymic progenitors also have self-renewing capacity[12], revealed following thymus transplantation into IL7R deficient hosts that lack common lymphoid progenitors, and it is possible this capacity may serve to ensure steady thymic repopulation and output in the face of inconsistent progenitor input.

Box 1: Transit and residence times in development

When estimating the time taken for cells to progress through development, care must be taken in the interpretation of the parameters of traditional ordinary differential equation (ODE) models. Take a population N that is fed at a constant rate θ from a precursor population, is at risk of death, and also matures to a downstream developmental stage. If maturation and cell death are both modelled as independent, first-order processes, then if μ is the *per capita* maturation rate and δ is the rate at which they die, then we can model the dynamics of the expected number of cells in this population using

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \theta - \mu N - \delta N. \qquad (1)$$

Here $\mu\,dt$ and $\delta\,dt$ are the probabilities that a cell will mature or die, respectively, within a short time interval dt. The *residence time* of a cell within this developmental stage – the time it spends there whether it matures or dies – is therefore exponentially distributed with mean $1/(\mu+\delta)$. However, the *transit time* – the time spent in the compartment by cells that successfully mature – follows the same distribution, also with mean $1/(\mu+\delta)$, and not $1/\mu$. Intuitively, if cells are at constant risk of dying, those that mature tend to do so earlier, on average, than those maturing at no risk of death. This apparent acceleration of maturation will be most apparent at developmental bottlenecks when death rates are substantial.

The DN3 stage represents a key checkpoint in development at which thymocytes that fail to undergo productive *Tcr* gene rearrangements die, and those that succeed commit to either an $\alpha\beta$ or $\gamma\delta$ lineage fate. While there are clues regarding the role of expression or signalling of the two prototypical TCRs in directing this lineage decision[13], the relative probability is unknown and is obscured by the distinct proliferative fates of cells following lineage commitment. In contrast to DN4 thymocytes, $\gamma\delta$ T cells do not undergo a burst of division and several distinct lineages of $\gamma\delta$ T cells emerge during ontogeny, defined by cytokine producing potential. IL-17 secreting dendritic epidermal $\gamma\delta$ T cells, that reside in the epidermis of the skin, are known to develop as a

distinct wave only in the fetal period[14]. In contrast, undifferentiated and interferon-producing $\gamma\delta$ T cells appear to develop in the thymus throughout life[14]. However, it is unclear what role this population has in the production and maintenance of peripheral $\gamma\delta$ T cells. In general we have little quantitative understanding of $\gamma\delta$ T cell repertoire development and homeostasis, and this area is open for modelling approaches.

Following successful *Tcrb* rearrangements, DN3 thymocytes undergo a proliferative burst accompanied by differentiation though the DN4 stage to generate the large pool of DP thymocytes that audition for selection and onward development. Modelling recovery of thymic development following drug induced depletion of cycling cells, Thomas-Vaslin *et al.*[8] estimated that thymocytes divide 8 times as they expand and transition from DN to mature DP, and that this flux replenishes up to a third of the mature DP pool every day. This estimate is in close agreement with BrdU labelling studies that found a similar fraction of DP thymocytes labelled with BrdU overnight[15] and our own studies of thymocyte development discussed below[16]. While it is not fully understood how this burst of cell division is controlled, there is evidence that an asymmetric cell division (ACD) following successful TCRβ-selection of DN3 cells may be involved[17]. Scribble is a factor required for ACD, and Scribble$^{-/-}$ mice have a subtly enlarged DN4 compartment. Ref. 17 used mathematical models to establish that DN3 cells likely undergo at least one symmetric cell division before an ACD event. Surprisingly, DP numbers are not altered in Scribble-deficient thymocytes, suggesting that expansion of DN4 into DP may involve a degree of quorum sensing rather than an autonomous program of divisions.

2.2 | Selecting the TCR$\alpha\beta$ repertoire

At the early DP stage, re-expression of the Rag recombinase complex allows rearrangement of *Tcra* genes so that DPs may attempt to express a mature $\alpha\beta$ TCR heterodimer. From this point, onward development of thymocytes is restricted to those expressing functionally relevant TCRs. Such cells are identified in different selection processes, governed by signals originating from newly expressed TCRs. A fundamental property of useful TCRs is that they have a low level of intrinsic recognition of self-MHC molecules that is thought to facilitate recognition of foreign antigens in the periphery[18]. Onward development of such cells is termed positive selection. Because of the random nature of somatic *Tcr* gene arrangements, many newly generated TCRs lack any intrinsic capacity to recognise self-MHC and fail to continue development, ultimately dying 'by neglect'. Others will recognise self-peptide MHC with high affinity and have the potential to be overtly autoreactive, and die through so-called negative selection. Precisely how the TCR repertoire subdivides into useful, useless or dangerous TCRs has been of great interest for many years. The low abundance of mature SPs, at around 5% of all thymocytes, established the view that a similar fraction of DPs express useful TCRs and undergo positive selection, and that a smaller fraction are autoreactive and are negatively selected, leaving the vast majority expressing useless receptors[2]. More recently, this question has been probed by different modelling approaches, discussed below, that suggest a far larger fraction of newly generated TCR are functional than previously thought.

Our own studies[16] took advantage of *Zap70*$^{-/-}$ mice, who lack expression of the tyrosine kinase Zap70 that is essential for TCR signalling. Without the capacity to transmit TCR signals, thymic development is arrested at the DP stage in these mice, but it is released following artificial restoration of Zap70 expression. This system revealed three dis-

tinct phases of DP thymocyte development, identified by expression of TCR and CD5 [19]. $TCR^{lo}CD5^{lo}$ DP1 thymocytes represent non-selecting cells. DP2 thymocytes are $TCR^{med}CD5^{hi}$ and include CD4 and CD8 lineage thymocytes at the earliest stage of selection (0-48hr after onset). DP3 thymocytes are $TCR^{hi}CD5^{med}$ DP cells that develop 48-72h following onset of selection, and are exclusively CD8 lineage cells. Using mathematical models to analyse the dynamics with which DP and SP compartments are restored provided new insights into the bottlenecks created by positive and negative selection. Sinclair *et al.* [16] estimated that ∼25% of DP1 thymocytes are positively selected but that extensive loss at DP2 and DP3 results in only ∼6% of DP1 thymocytes making the transition to SP. This high failure rate suggested that the extent of negative selection during the DP phase is far higher than had previously been thought. Analysis of $Bim^{-/-}$ mice supports this conclusion [20]. Bim is a pro-apoptotic protein required for induction of cell death during negative selection, and its absence leads to the accumulation of a large population of post selection DP thymocytes (defined as $CD69^{+}$ DPs that include both DP2 and DP3 subsets) that apparently receive strong TCR signals, as measured by Nur77 EGFP reporter expression. Subsequent modelling of development in wild-type and $Bim^{-/-}$ mice in the steady state arrived at similar conclusions to those derived from our analysis of inducible development in $Zap70^{-/-}$ mice – that 35% of DPs are positively selected but that as many as 92% of these fail by negative selection at the late DP stage [21].

Both Sawicka *et al.* [21] and Sinclair *et al.* [16] concluded that the fraction of SPs that die is lower than the proportion lost at the late DP stages (8% or 20% of CD4 SP respectively, and 32% or 42% of CD8 SP respectively). Therefore it appears that negative selection may occur in two waves. A first large wave during the DP stage, presumably in the thymic cortex and/or during migration to the medulla, purges the repertoire of grossly autoreactive cells, of which there are many. A second wave occurs amongst SP thymocytes and results in deletion of far fewer cells, but likely represents a fine tuning of the repertoire to eliminate rarer thymocytes specific for autoantigens expressed on medullary epithelial cells. This latter process is critical for self tolerance [22,23]. Overall, negative selection is therefore a costly process. Because the TCR is cross-reactive, intuitively the stringency of negative selection is shaped by the needs to maintain coverage of the space of foreign peptide-MHC ligands and to avoid reactivity to self. Strikingly, theoretical studies of this trade-off have yielded estimates of the optimal rate of negative selection that are remarkably close to the more empirical estimates described above (see ref. 24 for a review).

2.3 | The emergence of the CD4:CD8 ratio in the thymus

The ratio in which CD4 and CD8 T cells emerge from the thymus derives in part from the lineage decision during the DP stage (see refs. 25,26 for reviews), which is influenced by the propensities of newly formed TCR to interact with MHC class I and II, and the subsequent population dynamics and stringencies of selection within each lineage. Models have helped to map how the CD4:CD8 asymmetry emerges. Sinclair *et al.* [16] used data from thymic development in MHC class I and class II-deficient mice to infer that roughly equal numbers of positively-selected DP1 thymocytes are class I and class-II restricted but that only ∼12% of class I-restricted cells in DP2 progress to SP8 while 45% of class II-restricted cell progress to SP4. These figures were validated by comparing the survival of class I- and class II-restricted DP thymocytes following adoptive transfer into normal thymi, and by demonstrating that class I-restricted cells were more susceptible to death through over-expression of pro-apototic factors. This skewing is consistent with estimates that 6% and 1% of DPs select into the single-positive CD4 and CD8 lineages [8]. In contrast, Sawicka *et al.* [21] inferred from steady-state thymi that the CD4:8 asymmetry emerges during continued negative selection

in the medulla, with greater loss of CD8 than CD4 SP (32% vs 8%) and a greater proportion of CD4 SP cells undergoing division than CD8 SP (46% vs 27%). While analysis of Zap70-induced development by Sinclair *et al.*[16] also suggested greater death amongst CD8 SP, it is less clear whether differential proliferation is a significant force favouring CD4 T cells. Thomas-Vaslin *et al.*[8] estimated that comparable fractions of CD4 and CD8 SP cells undergo one or two divisions, a conclusion supported empirically[16].

While there is clear evidence that a far larger fraction of Class I- than Class II-restricted thymocytes die during selection, whether this is exclusively the result of more stringent negative selection is less clear. Triggering CD8 lineage development is associated with weaker or more transient TCR signals than CD4 development[19,27,28], and so it seems counterintuitive that CD8 lineage cells would be more susceptible to negative selection. On one hand, the death rate estimates of Sawicka *et al.*[21] were in $Bim^{-/-}$ mice, and therefore perhaps constitute an explicit measurement of the effect of negative selection. On the other hand, thymocytes require continued TCR signalling for survival[29], so it is also possible that death of class I-restricted DP thymocytes arises in part because weak self-recognition was sufficient to initiate, but not complete, the process of positive selection. In this regard, measuring levels of negative selection in class I- or class II-deficient Bim-deficient hosts would help distinguish these possibilities.

2.4 | Post-selection maturation and regulatory T cell development

Modelling has estimated that SP4 and SP8 cells reside for 5-6 days and 4-6 days respectively[8,16], consistent with prior empirical estimates [19,30]. This residence includes the later stages of negative selection, as described above, 1-2 divisions[16,21,31], and further differentation before cells are mature enough for export. SPs acquire the capacity to proliferate in response to TCR triggering and induce expression of surface receptors that permit lymphocytes to start recirculation, such as S1P1 receptor and L-selectin (reviewed in ref. 32).

Foxp3-expressing $CD4^+$ regulatory T cells (T_{reg}) are also crucial for maintaining self tolerance and are induced at the CD4 SP stage in a process that takes 3-4 days[33,34]. The mechanistic basis of the lineage decision is still unclear but it requires TCR signalling, and T_{reg} are thought to to be cells with relatively high self-reactivity, close to the threshold of negative selection[35]. Relatively few modelling studies have explored T_{reg} development and we do not review the literature here, but a study of ours was motivated by a striking experimental finding that cohorts of T cells expressing the same transgenic TCR differentiate into both conventional and regulatory cells in the same environment[36]. At increasing levels of availability of the TCR's agonist peptide-MHC, T_{reg} differentiation was progressively more favoured until numbers of both lineages decreased at high peptide densities, presumably due to heightening levels of negative selection. These observations imply there is a strong stochastic element to the TCR-mediated component of the conventional/regulatory T cell fate decision in the thymus. Bains et al.[37] applied a probabilistic model to data from this study to probe the mechanisms by which developing T cells integrate information from TCR interactions to make fate decisions. Using a simple graphical argument, together with the information that TCR sensitivity changes progressively during development (see ref. 37 and references therein) they inferred that commitment can be triggered by extremely low numbers of TCR-peptide-MHC interactions, which lead to T_{reg} commitment if encountered while TCR sensitivity is low but deletion (negative selection) if encountered when TCR sensitivity is higher. The model shows that one does not need to invoke a need for qualitatively different signals for conventional and T_{reg} development, and also explains apparently paradoxical observations regarding the effect of partial and full

TCR agonists on the efficiency of T_{reg} production[38]. Such specificity in the fate decision likely assists in the generation of tolerance to self antigens without excessive deletion of the repertoire.

3 |Recent thymic emigrants

Although maturation of SP thymocytes is critical for their export, it appears that CD4 and CD8 T cells continue to develop even after leaving the thymus as recent thymic emigrants (RTE) and are distinct from mature naive cells[39]. Much of our understanding of RTE biology comes from Rag2-EGFP transgenic mice, in which green fluorescent protein (GFP) expression driven from a Rag2 promoter persists in newly developed T cells for as long as 3 weeks [40]. The GFP system has revealed subtle increases in the expression of IL-7R, Qa2 and CD28 over this timeframe, although these differences are insufficient to distinguish RTE from the rest of the naive compartment. Thymocytes whose egress is prevented with the S1P agonist FTY720 continue maturation normally (Sinclair and Seddon, unpublished observations). Thus the ligands responsible for this maturation, which include type I interferons, TNF and CD70 and exert their effect through NFκ-B signalling[41], must be present in both thymic and peripheral lymphoid tissues. RTE maturation therefore likely represents a continuation of processes that begin late in development in the thymus.

Functionally, RTE undergo weaker proliferative responses and secrete lower levels of effector cytokines following TCR stimulation[40]. The reduced expression of IL-7R by RTE in mice may impact their ability to join the mature pool, since T cells that fail to upregulate IL-7R exhibit reduced survival and reduced ability to undergo homeostatic cell division[42]. Therefore, RTE may be at competitive disadvantage compared with mature naive T cells. It remains an outstanding problem to establish the lifespan of RTE relative to mature naive cells and their rate of maturation. Measuring these quantities is important because the TCR diversity of the naive reportoire can only be increased by the release of new T cells from the thymus, and so we want to understand how rapidly and efficiently these cells join the mature naive pool.

In mice, our knowledge of RTE dynamics comes from following the fates of newly-exported cells identified using either (i) the Rag2-EGFP system, (ii) a congenic marker expressed on adoptively transferred or engrafted thymocytes, or (iii) division-linked DNA labelling. The latter is most useful in mice, in which thymocytes proliferate substantially but peripheral naive cells divide rarely, and so over short periods the naive T cells that have accrued label can be inferred to be enriched for RTE[43].

Two important studies[44,45] examined RTE and mature naive T cell homeostasis by transplanting additional thymi into healthy mice and quantifying the kinetics of the host and donor-derived naive CD3+ (that is, combined CD4 and CD8 naive T cell) populations. The accumulation of donor cells in the peripheral pool was close to the estimated total number of cells exported from these thymi in the previous three weeks[45], and the donor derived T cells were lost rapidly three to four weeks after transplantation[44], when donor T cell production ceased due to repopulation of the grafted thymi with host-derived thymocytes. The authors inferred that RTE lived for approximately three weeks and during this time were transiently exempt from homeostatic regulation, but the kinetic of their accumulation and loss could also be explained with RTE having a relatively narrow distribution of times to die. Broadly, the behaviour they observed is consistent with a simple model of the flow from thymus to RTE to mature naive T cells, with maturation and loss occurring in both cell populations at random. An RTE lifetime of three weeks is slightly shorter than most estimates of the population-average lifetimes of naive CD4 and CD8 T cells in mice[6,46,47]. However, later studies that tracked RTE and mature naive cells transferred into the same mouse have come to opposite conclu-

sions regarding their relative abilities to survive[48,49]. These latter studies were performed in mice of different ages, and as discussed below the survival or proliferative ability of naive T cells likely changes with host and/or cell age. This effect may be strong enough to significantly and progressively alter the ratio of RTE lifetime to the population-average lifetime of mature naive cells as the mouse ages. A recent study[50] modelled data from thymectomy, thymus transplantation, and from several deuterium labelling studies, all in mice aged around 12 weeks. Fitting models simultaneously to all three datasets, they also concluded that CD4 RTE have an expected lifespan of about 3 weeks, less than that of mature naive cells. They also estimated that the expected time for a CD4 RTE to mature is about 8.5 weeks, meaning that less than a third of them become fully functional naive cells. In contrast, van Hoeven *et al.*[50] did not detect a difference in lifespan between CD8 RTE and mature naive CD8 T cells.

Thomas-Vaslin *et al.*[8] modelled both intrathymic development, as discussed above, and also maturation and homeostasis of naive T cells, by following cell numbers following transient depletion of dividing cells in both euthymic and thymectomised mice. They inferred that the naive compartment comprises dividing RTE undergoing a conveyor-belt sequence of two divisions, with a mean residence time of a few days, and resting, long-lived cells in roughly equal proportions. They estimate that naive T cell production through proliferation, which is almost exclusively within RTE in their model, is three times higher than the rate of production from the thymus, which is at odds with other estimates in which thymic export dominates over peripheral production[6,43,47]. It seems likely that this discrepancy may be due to increased homeostatic proliferation following the depletion treatment, and so these dynamics are probably not reflective of RTE behaviour at steady state.

We have a relatively limited understanding of RTE dynamics in humans, in large part because their contribution to total peripheral production is small[47] but also because, as in mice, we lack definitive phenotypic markers. A subset of naive T cells expressing CD31 is rich in T cell receptor excision circles (TRECs), non-replicating fragments of DNA that are by-products of the generation of the T cell receptor[51] and diluted within a population by cell division. The TREC content of CD31+ naive cells declines only slowly with age as thymic output falls, suggesting that CD31+ cells are rich in RTE, and the marker tends to be lost following homeostatic division[52]. However this process is not complete[53], meaning that subsets of CD31+ positive cells and their offspring may have been resident in the periphery for some time. In line with this, Bains *et al.*[54] used a small dataset from healthy and thymectomised humans with a survival analysis model in which RTE maturation is linked to post-thymic cell age, to infer that there may be considerable heterogeneity in the rates of maturation of RTE, defined by the loss of expression of another putative RTE marker, protein tyrosine kinase 7 (PTK7). Taking a different approach to inferring RTE dynamics, Vrisekoop *et al.*[43] used heavy water labelling in human volunteers to study replenishment and turnover of naive T cells and found that, strikingly, labelled naive T cells were lost extremely slowly over the 16 weeks following withdrawal of label. Their initial interpretation was that recently-produced (labelled) cells are more long-lived than the average and so RTE and naive cells produced by homeostatic proliferation are preferentially incorporated into the naive pool. The authors later showed that the most parsimonious explanation of these observations is that naive T cells simply form a single homogeneous population of long lived cells[55], and that because uptake of label is slow it is difficult to make any inference about the relative lifetimes of RTE and mature cells, or to estimate the efficiency with which RTE are incorporated into the mature pool. A later study by the same group[56] similarly found no signal of heterogeneity in turnover within the naive T cell compartments in humans. It seems likely that only direct

identification of RTE will allow us to quantify their dynamics in humans under replete conditions.

4 |Mature naive T cells

4.1 |The population dynamics of naive T cells in mice

The rate at which new naive T cells are exported from the mouse thymus into peripheral circulation is typically assumed to be directly proportional to total thymocyte numbers. This rate of export rises from birth, peaks near 8 weeks of age and then declines exponentially, halving roughly every 150 days[6,57,58]. Once in the periphery, naive T cells also undergo proliferative renewal in both mice and humans. In mice it occurs with a slow kinetic that is consistent with entry into cell cycle being a Poisson process[6]. This mode of renewal through single divisions is also observed in memory T cells[59,60], and contrasts with the rapid and more deterministic program of divisions that takes place during antigen-driven clonal expansion of naive T cells into effector and memory populations[61–63].

The long-term dynamics of the naive T cell pools in healthy adult mice can be described remarkably well by a model pairing a declining thymic source with constant rates of division and death,

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \theta_0 e^{-\nu t} - \lambda N, \qquad (2)$$

where λ is the net effect of of loss of naive cells through death or differentiation and cell division[47,64]. Ki67 is a nuclear protein that is detectable for 3-4 days following cell division[55,60,65,66], and is detectable in roughly 4% of naive CD4 and CD8 T cells in adult mice[6]. This level gives an upper bound on the rate of homeostatic division of roughly (0.04/3.5)/d, or a mean interdivision time of at least 100 days. This estimate will increase if the Ki67 fraction includes any residual expression from intrathymic proliferation. The expected residence times of naive CD4 and CD8 T cells (the average time taken after thymic export to leave the naive pool due to loss or differentiation) are 2 or 3-fold shorter than this[6,47]. Because total naive T cell numbers in mice fall only by a factor of two between 100 and 500 days of age[47,64], this simple analysis confirms that naive cells in adult mice are sustained largely by thymic export[47].

This simple model implies that naive T cells are ignorant of each other, but in other physiological settings there are multiple strands of evidence for competition or quorum sensing. Naive T cell numbers in thymectomised mice decline more slowly than expected from equation 2 (ref. 47). That study concluded that either cell loss rates decreased or division rates increased as numbers fell, due to an increase in the availability of homeostatic stimuli. Indeed other studies have assumed competition among naive T cells in mice, modelling it as a simple carrying capacity encoded as a density-dependent rate of proliferation or loss[67,68].

Such models are perhaps motivated by the observation that naive T cells transferred to severely lymphopenic mice, or those emerging into the periphery following T cell depletion, proliferate much more rapidly than in replete conditions[8,69–71], with a mean interdivision time of hours or days[8,72,73], and this lymphopenia-induced proliferation (LIP) appears to slow as the naive compartment fills[72]. It seems likely that this slowing is due at least in part to increasing competition for resources, because the fold expansion is inversely proportional to the number of cells transferred[73,74]. The extent to which resource-competition limits homeostatic division under normal conditions, however, is unclear. In one study in mice, more than 90% of peripheral T cells had to be depleted before LIP was observed[75] and levels of homeostatic proliferation as measured by Ki67 show very little change with mouse age as cell numbers fall due to waning thymic output (T. Hogan and B. Seddon, unpublished observations) or even following thymectomy[43].

The neonatal mouse environment might be considered lymphopenic and indeed supports the proliferation of adoptively transferred naive T cells from adult mice[76], but the expansion observed in that study was accompanied by a transition to a memory-like phenotype and so cannot be the mechanism of accumulation of naive T cells during the first few weeks of life. Further, while naive T cell Ki67 levels are higher in neonates than in adults, SP thymocytes are also more proliferative early in life (T. Hogan and B. Seddon, unpublished observations). It is therefore unclear to what extent the rapid accumulation of naive T cells in very young mice is driven by a mode of lymphopenia-induced proliferation which preserves a naive phenotype, or from a highly active thymus, with associated residual expression of Ki67 deriving from the last stages of thymic development.

Understanding the nature of resource competition among naive T cells is complicated by their non-redundant requirements for both TCR signals[77–80] and cytokines such as IL-7[79,81,82]. TCR signals in CD8 and CD4 T cells derive from contact with cells presenting self-peptides in the context of Major Histocompatibility Complexes (self-pMHC-I and self-pMHC-II respectively)[83–86]. Determining the necessity of TCR interactions in homeostasis has historically been difficult due to the complexities of fully ablating MHC but a consensus has emerged that TCR signals are required for naive T cell survival under healthy conditions[80]. Cytokines such as IL-7 are produced by stromal cell components of primary and secondary lymphoid organs, such as follicular reticular cells[87] and lymphatic endothelia[88]. Naive CD8 T cells can additionally take advantage of IL-15 to support both their survival and proliferation under lymphopenic conditions[89]. The overall size of the T cell compartment appears to be influenced by the abundance of IL-7, with over-expression of IL-7 leading to an increase in total peripheral numbers that is driven by changes in peripheral dynamics and not increased thymic output[90]. For CD4 T cells this increase is manifest in both naive and memory compartments, but the increase in CD8 T cell numbers is in memory only, perhaps in part due to conversion of naive T cells[91].

LIP of naive CD4 and CD8 cells is driven by both TCR signaling[92,93] and IL-7[82]. There is also evidence that cells' interpretation of homeostatic signals is subject to dynamic tuning. Sensitivity to self-pMHC may be controlled dynamically by CD5, a negative regulator of TCR signaling[94] which itself may be under feedback control from TCR signals[79,95], and CD8 T cells deprived of self-pMHC class I exhibit increased sensitivity to TCR stimulation[96]. Similarly, IL-7 signaling may feed back to inhibit expression of the IL-7 receptor as an 'altruistic' response to homeostatic cytokine signaling[97]. In humans, the role of cytokines such as IL-7 and IL-15 for survival of naive cells is well established[98]. Whether TCR signals tune functional activity of naive T cells and promote their survival in a similar manner to that described in mice is not known, as we lack an appropriate experimental framework to investigate such signalling in vivo in humans.

Together these results suggest that naive T cell numbers are regulated through the availability of shared resources. This quorum-sensing is mediated by the interplay of at least two signals whose availabilities likely become limiting at different cell densities, and have differing impacts on survival and the propensity for proliferative renewal. It seems that competition for these signals predominantly tunes survival at or near normal cell numbers, but these stimuli drive proliferation when not limiting. We return to the issue of the 'public' or TCR-clonotype-specific nature of MHC-derived stimuli below.

4.2 |Heterogeneity in naive T cell population dynamics

To add to this complexity, several experimental observations regarding naive T cell population dynamics cannot be explained purely with quorum-sensing models, which implicitly assume that all cells have the same rates of division and of loss at any given

time. One is that aged naive cells in mice appear to have a survival advantage over younger naive cells in the same environment[99]. Another derives from an experimental system using mice in which lymphocyte precursors in the bone marrow are replaced with congenically labelled counterparts following treatment with the transplant conditioning drug busulfan, which depletes stem cells while leaving the thymus and periphery intact[6]. Monitoring the replacement of host cells with new donor-derived T cells into the peripheral T cell compartments allows one to follow the fates of cell populations of different ages and so to test different models of homeostatic renewal and replacement. For the case of a single, homogeneous population maintained at constant numbers, one would expect donor cells to gradually replace host cells to a stable level equal to the chimerism achieved in the upstream (progenitor) population, on a timescale determined by the rate of population turnover. However, in adult recipient mice donor-derived T cells populate the mature naive CD4 and CD8 T cell compartments to only 80-90% of the level expected (*i.e.* of the chimerism attained amongst naive precursors within the thymus)[6]. Replacement is complete in other populations such as B-cells and naive $\gamma\delta$ T cells (T. Hogan, M. Verheijen, B. Seddon, unpublished observations), indicating that the incomplete replacement of naive $\alpha\beta$ T cells is not an artefact of the experimental system. One potential explanation of the shortfall is that the normal decline in thymic output with mouse age causes the influx of donor cells to dwindle before the chimerism in the mature naive pool can reach that in the thymus, which is established within a few weeks after bone marrow transplant. However, even for a general homogeneous birth-death models with rates of loss and division varying arbitrarily with time, thymic involution is too slow to explain the incomplete replacement[6]. The difference in the average behaviour of host- and donor-derived cells, together with the increased fitness of older cells[99], then argues against purely homogeneous, potentially resource-limited models of turnover in which all naive cells are equally likely to divide or die within any given time interval.

Heterogeneity in homeostatic dynamics could derive from multiple sources. There may be stable phenotypic variation – that is, the naive pools comprise subpopulations with different rates of turnover that occupy distinct homeostatic niches. A putative population of host-derived 'incumbent' cells, established early in life and resistant to displacement, was invoked to explain the incomplete replacement of naive T cells in the busulfan chimera system[6]. Heterogeneity could also emerge progressively through selection or adaptation. In a pure selection scenario, natural variation in the fitness of cells exported from the thymus[64,100,101] generates heterogeneity in the mature naive T cell pool which develops over an individual's lifetime through the accumulation of longer-lived or more proliferative cells. If this fitness distribution derives from cell-cell variation in the average affinity of the TCR for self peptide-MHC[95,102], the naive pool may become progressively enriched for strongly self-reactive cells, potentially increasing the risk of autoimmune disease with age[18]. The rate at which any selection occurs will be magnified by the gradual decline in thymic output, which progressively starves the pool of new TCR specificities. In a purely adaptive scenario, naive cells are born equal and the dominant source of heterogeneity is cell age; as cells spend more time in the periphery, their fitness changes relative to younger cells in the same environment, either deterministically or stochastically through the accrual of mutations[103]. The distribution of fitnesses under selection or adaption might be shaped further through additional competition for resources, either globally or within TCR-specific niches.

Without tracking the fitness of individual cells with age, these potential sources of heterogeneity are difficult to distinguish experimentally. For example, Tsukamoto et al.[99] ascribed the apparent increase in lifespan of old cells to a process of adaptation or conditioning, and not selection. They argued that given the natural decline in thymic output and the short

average lifespan of naive cells in young adult mice (∼4-6 weeks for naive CD4, ∼8-11 weeks for naive CD8[6,47,83]) selection for long-lived cells should be complete in middle aged mice; yet they saw a continuous increase in fitness of cells taken from mice aged between 6 and 24 months, relative to cells in younger animals. However, with its approximately exponential decline with a half-life of roughly 6 months[6], thymic output is still appreciable in 2 year-old mice and so selection may well continue to operate into old age. This uncertainty highlights how quantitative models are potentially very useful for discriminating between candidate biological mechanisms.

One can assess the support for different models using statistical criteria, but another test of a model's strength is its ability to explain multiple independent sets of observations. Taking this approach, a recent study of ours[64] compared a suite of candidate models of naive T cell homeostasis (Figure 2), describing constant rates of division and loss (equation 1), density-dependent rates of division or death, adaptation, selection, and population heterogeneity with incumbent cells. We confronted these models with three datasets relating to naive T cell homeostasis under healthy conditions or in partial lymphopenia; the kinetics of T cell numbers in both euthymic and thymectomised mice reported by den Braber *et al.*[47], the kinetics of naive T cell replacement in busulfan chimeras of different ages, and the results of adoptive transfers of naive CD4 T cells from hosts of different ages, reported in Tsukamoto *et al.*[99]. Only the adaptation model was able to simultaneously explain all three datasets (Figure 2), with fitness increasing slowly on a timescale of roughly 100 days. This pace of accrual of fitness is somewhat at odds with the shorter timescales of RTE maturation, and indeed a model of a conveyor-belt mechanism of RTE dynamics, a special case of adaptation in which all cells progress to maturity (and higher fitness) after a fixed time in the periphery, explains these diverse datasets poorly[64]. The study also supports the conclusion of Tsukamoto *et al.*[99] that selection alone is unable to explain the trend in naive T cell survival with host age.

This strong support for a dominant role for adaptation in naive T cell homeostasis drew on the principle of parsimony, but other homeostatic mechanisms likely operate, to different extents. In particular, as discussed above, resource competition likely regulates cell numbers as thymic output declines, although it seems this may be a relatively weak effect and only apparent under more extreme physiological perturbations. This uncertainty highlights the challenge of characterizing complexity in biological systems. Multiple mechanisms likely operate, but our ability to identify and parameterize them all simultaneously in a single unified model is limited by the number of datasets available and our ability to reliably search high-dimensional parameter spaces for the best-fitting predictions.

4.3 | Regulation of naive T cell numbers in humans

There is equivocal evidence for regulation of naive T cell numbers through resource competition in humans. A study by Dutilh and de Boer[104] used TREC measurements to infer the existence of a density-dependent homeostatic mechanism – that the net rate of loss of naive T cells in humans is positively correlated with cell numbers. In homogeneous models of naive T cell turnover the TREC frequency – the average number of TRECs per T cell – is unaffected by cell death and is instead determined by the influx of TREC-rich cells from the thymus and their dilution through cell division. (Conversely, the absolute number of TRECs in an individual reflects influx and loss but not cell division, and the number of TRECs per unit volume of blood may be a useful correlate of thymic output[105].) Dutilh and de Boer showed that the age-related decline in TREC frequencies in healthy humans was too rapid to be explained by thymic involution alone. They argued that the shortfall could be explained most simply by a compensatory increase in homeostatic cell division

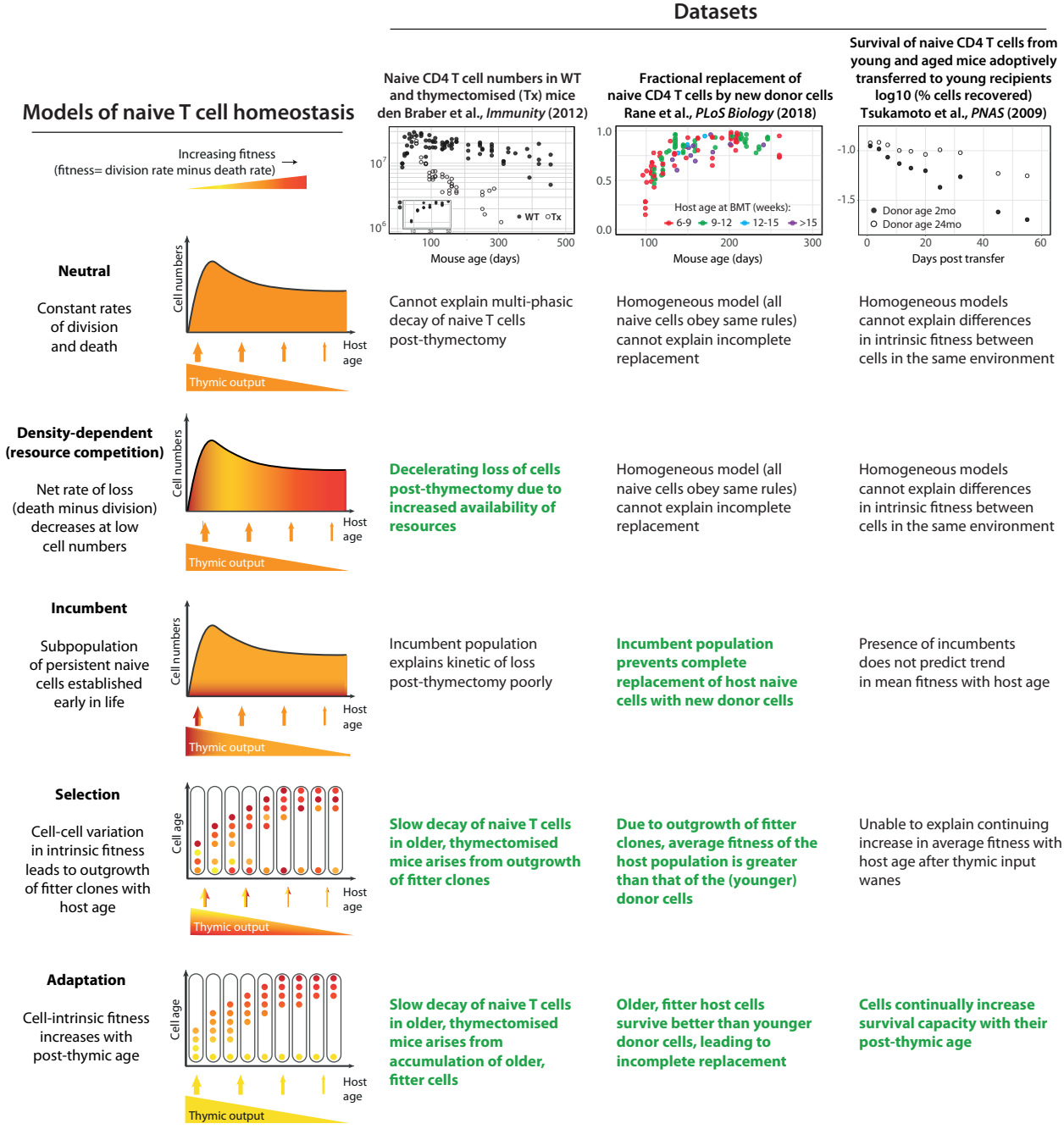

Figure 2: Comparing the ability of multiple models of naive T cell homeostasis to explain diverse datasets. Green, bold text indicates that the model is able to explain the corresponding dataset. Only the adaptation model is able to explain all three sets of data alone. (Figure adapted from ref. 64).

driven by the (modest) decrease in naive cell numbers with age. Indeed one study has shown an increase in homeostatic division and decline in TRECs per PBMC after 20 years of age [106]. One explanation for the discrepancy is that there were differences in the ages of the individuals in each of the studies. Another explanation is the one pointed out by Dutilh and de Boer, that the TREC dynamics could also be explained by increased cell survival rather than an increase in cell division with age. In this scenario TRECs are slowly degraded within cells, and any increase in cell lifespan with host age will expose this loss and reduce TREC frequencies further. Detecting changes in cell survival would be difficult to quantify using division-labelling alone, if one relaxes the assumption that the population is in perfect equilibrium with division balancing death. Indeed any of these effects may be small, because modelling of deuterium labelling in young and old adult humans revealed surprisingly little change in the rates of division or turnover of naive CD4 and CD8 T cells with age, as their numbers fall and thymic output wanes [107].

Nevertheless, Reynolds *et al.* [108] modelled naive T cell homeostasis in humans assuming that quorum-sensing does operate under normal conditions, mediated by competition for a finite resource of IL-7 that regulates both cell survival and homeostatic proliferation. In this model cells possess different IL-7 signalling thresholds for the two processes, which are both lognormally distributed across the population. Depending on the probability of survival (p) immediately following cell division, the model predicts either a unique stable compartment size ($p > 0.5$) or if $p < 0.5$ stability is followed by a bifurcation and subsequent crash in the naive T cell numbers in early adulthood, at an age determined by the decline in thymic output and a saturation in the rate of production of IL-7 (assumed proportional to body mass). This model predicted an upper bound on the proportion of naive cells in cycle of 0.05%. Approximately $0.2 - 1\%$ of naive T cells are Ki67+ in young adult humans [43,109]. Assuming a cell cycle duration of 12h [108] and a Ki67 lifetime of 3.5 days, these observations imply that 0.03-0.14% of naive cells are in cell cycle at any time, which is in broad agreement with the predictions of Reynolds *et al.*.

In contrast, there is strong evidence that homeostatic proliferation contributes substantially to the reconstitution of the T cell compartments following therapeutic depletion [110] and is manifest in the lymphopenia induced by untreated HIV infection [111]. Such polyclonal proliferation is commonly associated with conversion of naive cells to a memory-like phenotype with effector characteristics, which if self-reactive likely contribute to autoimmune diseases [110].

4.4 | Estimating the relative contributions of thymic output and peripheral division in humans

One of the key differences between mice and humans with regard to T cell homeostasis is the relative contribution of thymic output and peripheral division to the maintenance of the mature naive T cell pools. The thymus in a young adult mouse releases of the order 10^6 cells per day [6,15,112,113], which is roughly 1% of peripheral T cell numbers and is 2- to 5-fold greater than the contribution of peripheral division to daily naive T cell production in adult mice [6]. Other estimates of this ratio are even higher [47]. In humans, however, the situation is reversed. Bains *et al.* [114] drew on several datasets (see references therein) indicating that TREC frequencies within naive CD4 T cell populations show very little change up to early adulthood in humans. Pairing this observation with a general model of naive T cell homeostasis which assumed a homogeneous naive compartment and allowing rates of cell division and loss to vary arbitrarily with time, they showed that peripheral production exceeds that of the thymus in

young humans:

$$\text{Production rate through division} = \left(\frac{c}{\tau} - 1\right)\theta(t) \simeq 2\theta(t) \quad (3)$$

where θ is the rate of export of cells from the thymus, $c \simeq 0.25$ is the average TREC content of a cell emerging from the thymus[115] and $\tau \simeq 0.08$ is the estimated (and roughly constant) frequency of TRECs per naive CD4 T cell in the periphery up to age 20 (ref. 111). If the naive T cell pool is assumed to be homogeneous, the predominance of peripheral division over supplementation from the thymus is the only conclusion that can be drawn from the large difference in the average TREC content of recent thymic emigrants and mature naive cells. An analogous argument was used by den Braber *et al.*[47], who made a very similar estimate of the TREC content of CD4+ RTE using SP thymocytes from thymectomised children, and estimated that post-thymic proliferation accounts for 5-7 times more production than thymic export in young adult humans.

Bains *et al.* also developed a formalism for direct estimation of thymic output using measurements of TREC frequencies and levels of Ki67 within mature naive cells[116]. Their formulation likely overestimates the level of thymic output roughly 7-fold, by using an estimated Ki67 lifetime that is too low (12h). Using the developed consensus of roughly 3.5 days shifts their estimate to ∼70 million cells per day in young adults, somewhat closer to the more recent estimate by den Braber *et al.*[47] of 16 million cells per day, based on a similar principle of combining measurements of TRECs and estimates of division rates using deuterium labelling.

As in mice, it is unclear to what extents the ontogeny of the naive T cell pool in infant humans is driven by thymic output and peripheral expansion. Schönland *et al.*[109] found that the frequencies of Ki67$^+$ cells in cord blood from third-trimester neonates of 30-40 weeks gestation were initially around 10%, roughly 100 fold higher than those in young adults, declining to ∼1% by 40 weeks gestational age. However during this period they also found that TREC frequencies within both naive CD4$^+$ and CD8$^+$ cells were stable, but also higher than seen in young adults. The simplest interpretation of these observations is that thymic output and post-thymic proliferation are both greatly elevated in neonates and fall in tandem. Due to the slow intracellular decay of Ki67, it is unclear whether this increased proliferation, which acts to reduce TREC frequencies, occurs in the periphery or late in thymic development.

4.5 | Are there TCR-specific niches in the naive T cell pools?

While there is clear evidence that TCR interactions with self-peptide-MHC (spMHC) ligands are implicated in naive T cell homeostasis, the specificity of these signals is unclear. If the necessary stimuli can be obtained with low-affinity binding, as they appear to be for positive selection in the thymus, these promiscuous ligands might be considered a common resource, and if access to them is limiting they mediate quorum sensing at the level of the total compartment size. In contrast, any degree of specificity in TCR-mediated homeostatic stimuli opens the door for a more complex picture of T cell homeostasis in which different TCR clones compete for access to diverse 'private' spMHC ligands. Due to the cross-reactivity of the TCR, such niches may be overlapping. Any such structure may facilitate tthe maintenance of TCR repertoire diversity.

Despite the rational basis and appeal of this mechanism, in mice there is relatively little direct experimental evidence for the existence of specific spMHC niches for T cell clones in the steady state, partly because many studies in this area have used TCR transgenic cells in unphysiologically high numbers or in lymphopenic settings. The expansions of different clonotypes are reduced when co-transferred to the same animal, suggesting a dominant role for a public resource[73] and intra-clonal inhibition has

been shown not to require interactions with MHC[117]. However, TCR specificity can impact the ability to obtain homeostatic stimuli[102,118], and TCR transgenic cells can be seen to receive weaker TCR signals in a monoclonal than in a polyclonal host mouse[119], although the compounding effect of competition for IL-7 makes it hard to assess whether survival is indeed impacted by these subtle changes in the TCR signalling. Perhaps the only study to directly address the possibility of TCR-specific niches using clonal frequencies closer to physiological levels is that of Hataye *et al.*[120], who demonstrated that the extent of proliferation of TCR transgenic T cells following transfer to normal mice was dependent on their clonal abundance.

The evidence for or against TCR-specific niches in humans is also limited, but to explore this issue Ciupe *et al.*[121] analysed data from patients with a profound defect in thymic development whose peripheral T cell compartments reconstituted following thymus transplantation. Their aim was to assess the relative importance of common and TCR-specific resources in regulating the size and TCR diversity of the peripheral T cell pool, essentially by comparing the rates at which these quantities reach equilibrium following transplant. They concluded that the carrying capacity for a single TCR clone in isolation is approximately 1000 times the typical clone size under normal (lymphoreplete) conditions, implying that in healthy individuals, T cell numbers are regulated far more strongly at the population level than at the clonal level. However, their study did not distinguish naive and memory T cells. The latter may contain more highly expanded clones and have a strong impact on diversity estimates. It therefore is possible that the equilibration of naive T cell diversity occurs on a different timescale to that of the peripheral T cell pool as a whole.

Assuming a significant role for spMHC niche-based competition, several studies have modelled the within-host evolution of TCR clonal structure using stochastic birth-death models. Lythe *et al.*[122] used a model of competition for a set of spMHC niches and estimate naive T cell clone sizes in humans to be of the order 10 cells, a result which is insensitive to the details of niche structure and level of TCR cross-reactivity by construction. This result assumes a 1:25 ratio of thymic output to peripheral production, which is rather low compared to experimental estimates, at least in young adults (see above). The estimated clone size would decrease if this ratio increases and so their analysis could quite reasonably be consistent with an average TCR clone size being close to one cell. Stirk *et al.*[123] described a model in which naive T cells are characterised by the degree to which their spMHC niche is shared by other cells, which they refer to as the mean niche overlap and might be identified with the cross-reactivity of the TCR. They show that all TCR clonotypes are guaranteed to go extinct within some finite time and that the lower a clone's niche overlap the longer its expected residence time, due to reduced competition for resources. They argue that clones with lower overlap in spMHC requirements likely have a correspondingly low coverage of foreign peptide-MHC, and so an intermediate mean niche overlap is optimal for maintaining a diverse, long-lived repertoire with effective coverage of the space of foreign epitopes. This result echoes the argument that there is an optimum level of cross-reactivity of the T cell receptor that results from a trade-off between the high specificity needed to avoid negative selection in the thymus or the periphery, and the low specificity (high cross reactivity) needed to increase the probability that the repertoire is able to recognise a given foreign pMHC[124]. In a subsequent study, Stirk *et al.* went further to derive extinction probabilities for clones as a function of their similarity in specificity[125].

4.6 | The onward journey – naive T cells constitutively generate memory cells

Naive T cells can be recruited into effector or memory populations through cognate pMHC interactions. Memory cells divide and turn over more rapidly than naive cells in both mice and humans and so they are more amenable to analysis using DNA labelling methods. We do not review these studies here but a key observation is that memory cells, even more so than naive cells, display considerable heterogeneity in their homeostatic dynamics. Such analyses typically assume that memory populations are self-renewing and at equilibrium, but the interpretation of label uptake kinetics, and estimates of rates of division and turnover, can be complicated or confounded if there is any influx into memory compartments. Recently it has been shown by us and others that even in the absence of overt infection, there are considerable constitutive flows from naive to memory in mice in addition to kinetic heterogeneity within both effector and central memory subsets[60,126]. Gossel *et al.*[60] studied the replacement of memory compartments following transplantation of busulfan treated adult mice with congenic bone marrow. Surprisingly, donor cells were observed to steadily infiltrate both central and effector memory compartments over the life of the hosts. In contrast to naive compartments, in which extensive replacement of host cells is observed, only around half of host memory cells are replaced by the donor influx. Nevertheless, analysing flow rates revealed the donor influx to be substantial, with ∼12% of CM and 6% of EM compartments replaced each week in young adult mice. Tonic flow into the memory pool was also reported by Kawabe *et al.*[126], following transfer of purified naive CD4 T cells into congenic hosts. They also addressed the key issue of the identity of the antigenic drivers for this memory influx, and identifed a role for self recognition. As described above, MHC-dependent proliferation of transferred naive T cells from adult mice generates memory CD4 T cells in neonates[76], and this force acting on neonatal T cells likely contributes to the early establishment of the memory pool in young mice. Significantly, Kawabe *et al.* showed that germ-free mice possess a CD4 memory compartment of comparable size to that in conventional SPF reared mice, suggesting that self-driven LIP is a major contributor to the establishment of the memory compartment. Similarly, they showed that conversion of naive to memory in adult mice was dependent upon TCR and CD28 signalling. Treatment of mice with broad-spectrum antibiotics did not reduce the extent of conversion to memory, and the authors argued that the same self-recognition drives this flow in both neonate and adult, albeit at different rates.

Although self recognition is one driver for establishing and feeding the memory pool in young mice, a study of mice co-housed with pet-store mice[127] revealed enlarged memory compartments, demonstrating that commensal and environmental microbes are also important drivers of the establishment, and potentially maintenance, of the memory compartments. It remains to be determined whether exposure to such antigens contributes to these tonic flows into memory, and what implications these flows – whatever their drivers – have for maintenance of preexisting memory to pathogens.

5 | Future directions and challenges

It is now clear that, at least in mice, the naive T cell compartment is far from a simple homogeneous pool of cells awaiting activation. Instead it is a complex mixture of cells at different developmental stages and of diverse ages, whose population structure shifts as the host ages and thymic output dwindles. It is also evident that diversity in both developmental status and residence in the periphery impacts T cells' functional and homeostatic properties. Mathematical approaches have played an important role in revealing

this complexity. They have also been successful in revealing the commonalities and differences in T cell homeostasis in experimental mouse models and humans, which would otherwise rely on distinct experimental analyses and approaches. There is also a considerable literature describing changes to the size, dynamics and TCR repertoire of naive CD4 and CD8 T cells that occur in old age[128,129]. We have not reviewed this literature but this is an area perhaps under-studied by modellers.

Precisely how heterogeneity in cellular homeostatic fitness becomes established, and its implications for the function of the T cell compartment as a whole remains to be fully elucidated. To what extent is diversity in fitness imposed during thymic development, and on which selection pressures act in the periphery to shape the T cell repertoire? Does the microenvironment play a role in tuning the behaviour of T cells? Or are there autonomous modifications to fitness as cells age? And what are the nature and structure of any homeostatic niches that underlie competition within and between clones for a place in the repertoire? Quantitative modelling approaches will continue to be important here, particularly as it seems likely that several such mechanisms may operate, and their combined effect may be difficult to predict intuitively.

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