Mathematical Modelling of T Cell Homeostasis

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I, Irenjeet Bains, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.
Abstract

T cell homeostasis describes the process through which the immune system regulates cell survival, proliferation, differentiation and death to maintain T cell numbers and diversity in a range of different conditions. The aim of this thesis is to better understand how this process leads to the development of the naive CD4$^+$ T cell compartment during childhood. Mathematical modelling is used in combination with experimental observations to estimate naive T cell kinetics over the lifetime of an individual. The analysis described here shows that post-thymic proliferation contributes more than double the number of cells entering the pool each day from the thymus. This ratio is preserved from birth to age 20 years; as the thymus involutes, the average time between naive T-cell divisions in the periphery lengthens with age and the naive population is maintained by improved naive cell survival. Thymic output is quantified from birth to age 60 years by combining models to interpret naive T cell TRECs and Ki67 expression data. Three distinct phases of thymic T cell output are identified: (i) increasing production from birth to age 1 year; (ii) steep decline to age 8 years; (iii) slow decline from age 8 years onwards. Finally, the role of inter-cellular variation in T cell residency times is explored. It is able to explain the persistence of PTK7$^+$ naive CD4$^+$ T cells in thymectomised individuals. Importantly, the model predicts the accumulation of veteran PTK7$^+$ T cells in older individuals and suggests that the residual population in thymectomised individuals will also consist predominantly of veteran PTK7$^+$ T cells. The model has implications for the use of PTK7 as a marker of recent thymic emigration and also naturally explains improved T cell survival in older individuals.
Publications & Abstracts


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Chapter 1

T cell immunology: an introduction

T lymphocytes form an integral part of the adaptive immune system. The adaptive immune system allows the body to mount a response that is specific to invading pathogen and confers long-term protection to re-infection with the same antigen. Each T cell expresses a receptor on its surface that enables it to recognise specific antigen-peptides in association with major histocompatibility (MHC) molecules. Together, the receptors on all lymphocytes cover a broad repertoire of antigens.

1.1 T cell development

The role of the thymus as the primary source of peripheral T lymphocytes was first discovered by Jacques Miller through the study of neonatally thymectomised mice [4]. Since then a huge body of work has further elucidated the process through which T cell precursors are produced in the bone marrow, develop in the thymus and enter the circulating pool as mature T cells. Thymic development encompasses extensive differentiation, division, T cell receptor (TCR) gene rearrangement and selection. Only a small fraction of thymocytes survive the thymic process and go on to enter the peripheral circulating population [5–7].
1.1 T cell development

The thymus is largely comprised of the thymic epithelial space (TES), lymphocytic perivascular space and non-lymphatic thymic tissue including fatty tissue, connective tissue and Hassall’s bodies. Thymopoiesis predominantly occurs in the TES; the TES can be divided into the cortex and medulla and ~90% of thymocytes are found in the TES at any given time [8]. Early lymphocyte progenitors migrate through the subcapsular zone of the thymus before entering the cortex. Extensive proliferation takes place in the outer cortex; the rearrangement of the TCR $\beta$ chain is followed by division and maturation. Thymocytes upregulate both CD4 and CD8 becoming double positive before undergoing rearrangement of the TCR $\alpha$ chain. Following this, double-positive $\alpha\beta$TCR$^+$CD4$^+$CD8$^+$ thymocytes migrate to the inner cortex where they undergo extensive positive selection; cells that are able to recognise self-peptides bound to major histocompatibility complex (MHC) ligands are saved from cell death while those that fail to achieve a sufficient threshold of TCR signalling will be lost through death by neglect [9]. Death by neglect is thought to account for the loss of 80-90% of thymocytes. The ultimate CD4 and CD8 lineage commitment of double-positive thymocytes is thought to depend on interactions with self-peptide-MHC complexes on cortical thymic epithelial cells (TECs) [10].

The chemokine receptor CCR7, expressed during positive selection, plays a role in guiding the migration of single positive thymocytes to the medulla [11]. Thymocytes spend about half of their lifespan in the medulla before entering the peripheral circulating lymphocyte population as mature naive T cells [12]. Negative selection is thought to predominantly occur in the medulla [13]; immature thymocytes that demonstrate too high an affinity to self peptide MHC are lost to apoptosis. The mature T cell repertoire is finely tuned by the interaction between thymocytes and MHC complexes to allow for the recognition of a diverse range of antigens while maintaining a high level of self tolerance; Daniels et al. [14] found that, while the negative selection threshold is sharply defined, positive selection appears to occur over a broader range of affinities (as illustrated in Figure 1.1). In the final stages of thymic development thymocytes
undergo a sequential change in the expression of several surface marker and acquire the ability to functionally respond to a variety of stimuli [15–18].

![Affinity model of thymic selection](figure1.png)

**Figure 1.1: Affinity model of thymic selection.** The affinity between the T cell receptor and self-peptide MHC complex determines the outcome of positive and negative selection and shapes the T cell repertoire (adapted from review by Klein et al. [10])

### 1.2 T cell function & activation

The role of a T cell is to recognise foreign antigen expressed on antigen presenting cells (APCs) in association with the MHC complex and initiate an appropriate response. The initial interaction between a T cell and antigen-MHC complex will typically occur in the secondary lymphoid organs. Each T cell has a different antigen-binding specificity and the number of T cells that can respond to a specific antigen is limited. As a result, the first stage of T lymphocyte activation involves clonal expansion of the relevant T cell receptor clone. Activated naive T cells undergo extensive proliferation and differentiation producing effector and memory T cells. The lineage relationship between naive, effector and memory T cells is not completely understood [19], however, it is widely accepted that antigen specific T cell populations can expand up to 1000-fold producing vast numbers of effector T cells that are able to destroy infected cells and release cytokines that stimulate B cells. Following the clearance of
infection, the majority of effector T cells are thought to be lost to apoptosis, while a small fraction of antigen specific T cells will persist in the memory T cell pool [20]. The intensity of this response and the size and function of memory cells induced will depend upon the strength of TCR activation, duration of exposure to the MHC-peptide complex, the extent of binding, co-stimulation, inflammatory factors, intrinsic expression of signalling proteins, possibly the proximity of other cells and environmental stimuli.

**Naive T Cell Activation**

Following export from the thymus, naive T cells continuously circulate between the secondary lymphoid organs and peripheral blood [21, 22]. One of the key difficulties of studying human T cell dynamics is that only 2% of lymphocytes are found in the peripheral blood at any given time [23]; however, studies have shown that nearly all lymphocytes will pass through the blood each day [21] (Figure 1.2). Within the secondary lymphoid organs, APCs, such as dendritic cells (DCs), macrophages or B lymphocytes, will present antigenic peptides to naive T cells. It is thought that a naive T cell will sample the peptides presented by APCs within a lymph node for several hours with the aim of encountering its specific antigen. Despite the complex architecture of lymph nodes, multi-photon microscopy studies reveal that naive T cells scan APCs in the lymph nodes through random movements and at relatively high speed. Each DC appears to interact with multiple T cells simultaneously, although, the duration of each T cell-DC interaction is thought to be short-lived [24]. If a cell fails to recognise its specific antigen it will return to the circulation through the efferent lymph vessel; on average, a typical naive T cell is thought to spend approximately half an hour in the circulation before homing to the next lymph node [25]. The recirculation is thought to guided by chemokine receptors and adhesion molecules such as CCR7, CXCR5 and CD31 [as reviewed by Andrian et al. [26]].

Antigen-proteins are bound to MHC proteins on the surface of APCs and these MHC-peptide complexes are able to bind antigen specific TCR/CD3 complexes
on T cells to form immune synapses and induce TCR signalling in activated naive T cells [27, 28]. The strength of T cell activation is regulated through a combination of TCR affinity, costimulation and inflammation. Naive CD4\(^+\) T cells require two signals to become activated; MHC binding with the TCR and secondary interaction between costimulatory molecules, expressed on the surface of activated APCs, and the CD28 coreceptor, expressed on the surface of CD4\(^+\) T cells. Costimulation is thought to enhance proliferation, cytokine production, cell survival, and the expression of CD40 ligands and adhesion molecules necessary for trafficking in response to antigen recognition [29]. Following successful activation by an immunogen naive T cells undergo clonal expansion and differentiation to produce a population of antigen-specific effector T cells. Activation driven by lower-affinity interactions with self-peptide MHC (spMHC) lead to a homeostatic response that is discussed later in section 1.3.

![Figure 1.2: Schematic of T cell distribution and trafficking between blood, secondary lymphoid organs and other tissues.](image-url)
Effector T Cells

Effector T cells represent a heterogeneous population with respect to their response to differing invading pathogens. The lineage commitment of successfully activated T cells depends on the cytokine environment, transcription factors, antigen load and costimulatory molecules present during stimulation. CD4+ effector T cells might differentiate into T-helper type 1 (Th1), type 2 (Th2), or type 17 (Th17) cells, defined by their distinct cytokine secretion profiles, or they might evolve into regulatory T cells which suppress T cell activation and mediate self-tolerance. Effector CD4+ T cells lack CCR7 which is required for lymph node homing and CD27 which is involved in T cell activation, and instead express a diverse range of homing molecules that allow them to migrate to the site of the infection.

Th1 cells are thought to mediate cellular immunity against intracellular pathogens including enhancing microbicidal activity by macrophages and cytotoxic cells [30,31]. Th1 cells are associated with high expression of interferon gamma (IFN-γ) and interleukin-2 (IL-2) and are regulated by transcription factor T-bet. In contrast, Th2 cells are chiefly involved in mediating humoral immunity against helminths and other extracellular pathogens. They help B cells to develop into antibody producing cells and recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation [30,31]. Th2 differentiation is thought to be controlled by transcription factor GATA-3 and Th2 cells are associated with expression and production of IL-4, IL-5, IL-9 and IL-13. Th17 cells are a population of CD4+ effector T cells characterised by their ability to produce IL-17, IL-22 and IL-23. Their primary function is thought to involve induction of tissue inflammation and immune responses against extracellular bacteria and fungi. Human naive T cells are polarised towards the Th17 phenotype by TGFβ, which induces master transcription factor retinoid-related orphan receptor (ROR)-γt, in combination with IL-6, IL-21 and IL-23 [32].

Effector T cell responses require strict regulation; excessive Th1 or Th17 responses can lead to organ-specific auto-immunity, whereas uncontrolled Th2
T cell function & activation

Responses are thought to play a role in allergies and asthma [31]. Regulatory CD4+ T cells (Tregs) enable immune tolerance by suppressing effector responses [33]. Antigen presentation by DCs that express IL-10 is thought to polarise activated T cells towards the formation of regulatory CD4+ T cells (Tregs) [31]. Tregs are typically identified by expression of forkhead box P3 (FOXP3); the transcription factor that plays a key role in their differentiation and function. Their immuno-suppressive function is dependent on signalling via cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a negative regulator of T cell activation, as well as secretion of the immune-suppressive cytokine, TGFβ (Read, 2000). In addition to Tregs that are induced from CD4+ T cells in the periphery, primarily in gut-associated lymphoid tissue (GALT), the Treg subset includes naturally occurring CD4+CD25+FOXP3+ Treg cells that develop in the thymus.

The lineage commitment of CD4+ T cells was originally thought to result in stable terminally differentiated cells; once a naive cell had polarised towards Th1 or Th2 phenotype it was thought that this phenotype could not be reversed, even when cells were exposed to conditions that promoted other effector lineages [34]. However, recent work suggests that the local micro-environment, through cytokine, cell-cell or cell-micro-organism contact, plays a role in directing and maintaining the lineage of effector T cells and it is not clear that such a clear distinction exists between different effector T cell types [as reviewed by Zhou et al. [31]]. T cells have been observed to co-express both Foxp3 and RORγt and it has been shown that Tregs have the ability to differentiate into Th17 cells [35]. Similarly, there is evidence to suggest that Th17 cells can convert to Th1 and Th2 cells in the presence of the appropriate cytokine inducers [36]. Most recently, it has been shown that Th2 cells can be reprogrammed to adopt an intermediate phenotype characterised by both Th1 and Th2 transcription factors, GATA-3 and T-bet [37], suggesting a more complex picture of T cell activation and differentiation than was previously assumed.
Memory T Cell Activation

A unique feature of the adaptive immune system is the ability to remember pathogens. Following infection, a small number of long-lived memory T cells are produced. Memory T cells provide long-term immunity against recurring antigens and can persist for decades; smallpox vaccination follow-up studies suggest that T cell immunity declines with a half-life of $\sim 8$-15 years and virus-specific memory CD4$^+$ and CD8$^+$ T cells were detectable up to 75 years after a single vaccination [38, 39]. Memory T cells are able to mount a more effective immune response than antigen-naive T cells; where effectiveness is measured by magnitude, speed and sensitivity of response to antigen. One aspect of this improved response follows from a higher frequency of antigen specific memory T cells; the naive T cell repertoire is more diverse than that of the memory T cell population, but as a result the number of cells that are specific to a given antigen will be smaller in the naive pool [40]. The memory response is typically faster and results in more vigorous expansion, although, the differences in the way in which naive and memory T cells respond to antigen encounter are not fully understood.

Memory T cells can be divided into central memory T cells (T$_{CM}$) and effector memory T cells (T$_{EM}$) with distinct properties. T$_{CM}$ maintain the ability to rapidly divide in response to secondary antigen activation, express both CD62L and CCR7 and circulate between the blood and secondary lymph nodes. On the other hand, it is thought that T$_{EM}$ do not undergo division in response to secondary infection, do not express CD62L nor CCR7 and are thought to migrate predominantly between the blood and peripheral tissues [41,42]. Further, T$_{EM}$ demonstrate effector functions, such as cytolytic activity and cytokine secretion, more rapidly than T$_{CM}$ on reactivation with antigen [43].

There are at least two different models for the way in which memory T cells develop. A popular model is that activated naive T cells give rise to a large number of effector T cells, the majority of which will be terminally differentiated and die, while a small number will further differentiate into the mem-
Figure 1.3: Possible models of T cell differentiation a: In the linear differentiation model the effector phase is a necessary step in memory T cell formation by activated T cells; b: The bifurcation model describes asymmetric division and differentiation of one activated naive T cell into both memory and effector states; c: The self-renewal model suggests that naive T cells differentiate directly into T_{CM}, T_{EM} cells migrate to site of inflammation and become terminally differentiated effector T cells. Diagram from review by Ahmed et al. [19]
ory T cell compartment and survive as long-lived memory T cells [40, 44]. An alternative to this linear T cell differentiation model is the idea that naive T cells may undergo asymmetric division upon antigen encounter, whereby one daughter leads to the production of effector cells and the other controls the production of a memory population. Evidence for this hypothesis comes from imaging studies of activated T cells undergoing their first division; Chang et al. [45] showed that several proteins, including IFN-γ, are unequally divided between the daughter cells leading to the production of either effector or memory T cells. Furthermore, studies of lymphopenia-induced proliferation have shown that memory generation can occur in the absence of antigen stimulation [46, 47], suggesting that it may not be necessary for naive T cells to pass through the effector phase in order to become memory T cells. The alternative models are described in Figure 1.3.

1.3 Maintaining T cell numbers

The basic definition of homeostasis is the ability of an organism to maintain equilibrium by adjusting its physiological processes. Evidence for homeostasis and tight regulation of the T cell population came from early studies demonstrating that the implantation of additional thymi, or removal of the thymic tissue, had a limited effect on the size of the peripheral T cell population [6, 48]. Moreover, the ability of the T cell population to reconstitute following immunodepletion further supports the notion that T cell expansion and survival is governed by some awareness of available "space" [49, 50].

T cell homeostasis involves the regulation of cell division, differentiation, survival and death to maintain stable T cell numbers and diversity. It is inevitably difficult to observe T cell homeostatic processes in most situations since the net effect of cell production and loss will be, by definition, negligible. A wide array of experimental techniques are used to estimate the rate at which the T cell population is turning over *in vivo* in healthy individuals, however, we still do not have a clear picture of how the immune system is able to manipulate thymic production, cell division and survival to maintain a functional T cell
population over the lifetime of an individual. The disruption of T cell equilib-
rium by thymectomy, immuno-depletion and subsequent reconstitution allows
us to gain deeper insight into the underlying homeostatic processes.

**Thymic output**

The thymus is known to involute with age, however, it has proven difficult to
quantify the number of cells that are exported each day and our knowledge
of the extent to which thymic production declines remains limited. Steinmann &
colleagues [8] conducted an extensive study of thymic tissue in individu-
als aged 0 to 107; although they observed no change in total thymic volume
with age, histological staining revealed an age-related decline in the relative
volume of the TES and a significant increase in the proportion of adipose tis-
sue. This strongly suggests that thymic production diminishes with age since
the TES is thought to be the primary site for thymopoeisis and contains \( \sim 90\% \)
of thymocytes. Inference from histological studies is limited, however, by the
assumption that size necessarily correlates to function, or that the number of
cells reflects output.

More than a decade ago, T cell receptor excision circles (TRECs) were identified
as a surrogate marker of thymic output [51,52]. TRECs are excised DNA rem-
nants that are produced as a by-product of TCR gene rearrangement within
the thymus and are thought to be highly conserved within T cells. Douek et
al. [52] showed that TRECs were most prevalent in children but diminished
with increasing age, in accord with a decline in thymic output. TREC content
was also shown to be significantly reduced in thymectomised and HIV-infected
subjects and correlated positively with naive T cell numbers. This work in-
spired a plethora of studies measuring TRECs to investigate the role of thymic
production in health and disease.

However, the same study by Douek et al. [52] showed that TREC concentration
was diluted by cellular proliferation since TRECs are not duplicated during
mitosis. As a result, the average TREC content per cell can be a misleading
measure of thymic function; a decline in TRECs is consistent with a decline in
thymic output, however, it might also reflect an increase in peripheral division. This was shown to be important by Hazenberg & colleagues [53] who reported that a decline in TREC in HIV infected individuals reflects increased proliferation rather than diminished thymic production. Later, mathematical studies showed that the concentration of TREC per ml, as opposed TREC per cell, would better reflect thymic output since the total number of TREC does not decline with division [54]. However, the use of TREC to quantify thymic output remains contentious since total TREC reflects both thymic production and the longevity of T cells; the contribution of T cell survival, which is likely to vary with age and in immunodeficiency, remains an unknown.

CD31 (platelet endothelial cell adhesion molecule-1) is a cell surface marker that is thought to identify two distinct subpopulations of naive CD4+ T cells with unique properties and TREC content [55]. The per-cell TREC content of CD31+ naive T cells is significantly higher than CD31- naive CD4+ T cells and since TREC content is diluted by peripheral expansion, CD31+ T cells are thought to have spent less time in the periphery than their CD31- counterparts.

CD31 is used as a surrogate marker for recent thymic emigration; studies have shown that the fraction of CD31+ naive CD4+ T cells drops from ~90% of all CD4+ T cells at birth to ~30% at age 60 years [55, 56]. This compares to a 10-fold drop in the number of TREC per CD4+ T cell over the same period [52], and a ~15-fold decline in the relative size of the thymic cortex [8]. As with TREC, the size of the CD31+ naive CD4+ T cell population reflects a combination of thymic output, proliferation and cell loss through death or change of phenotype. In order to accurately interpret the data quantitatively we require an understanding of how naive cells divide and differentiate in-vivo and the extent to which cells may proliferate without losing the CD31 marker [56].

Peripheral turnover

In vivo isotope labelling studies are commonly used to study the turnover of T cells in both healthy and HIV-infected individuals [57–62]. A stable, isotopically enriched precursor is administered to subjects and is then incorporated
into the newly synthesised DNA of dividing cells. The number of labelled cells is recorded over a series of timepoints during and post labelling and is a measure of the cell division. Mathematical models are used to interpret the labelling data and infer the underlying rates of T cell production and loss.

Deuterium labelling, both deuterated glucose and heavy water, works through the administration of a non-radioactive, stable, isotopically enriched precursor which labels the glucose in newly synthesised DNA. As opposed to BrdU, deuterium is incorporated into DNA through the \textit{de-novo} nucleotide synthesis pathway. The enzymes of this pathway are up-regulated during S phase, hence the efficiency of this pathway is thought to be high and constant in dividing cells. It is also relatively unaffected by extracellular nucleoside concentrations. BrdU is a pyrimidine nucleoside that is incorporated into DNA through the nucleoside salvage pathway, as a thymidine analogue. This is not widely used in humans because of toxicity. In addition, the efficiency of BrdU incorporation is unknown and depends on the availability of extracellular nucleosides. BrdU labelling is also more complex to interpret since division leads to weaker labelling and the decay of label is artificially accelerated by cells reaching a division threshold in addition to cell loss. The impact of this depends on how many times a cell is likely to divide within the short labelling period. In contrast, during \textsuperscript{2}H glucose labelling only cell death results in loss of label.

Mohri and colleagues [59] originally noted that the rate of both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell production as measured by deuterated glucose labelling in healthy humans was considerably smaller than the rate of loss. Since the size of the lymphocyte population was known to be approximately stable it was assumed that the extra loss must be compensated for by the additional production of unlabelled, or equivalently not recently divided, T cells from the thymus. An alternative interpretation was later proposed by Asquith et al. [63] who identified that labelling studies measure the rate at which label is acquired by the entire T cell population, whereas, the rate of loss is only measured in recently divided T cells. It was argued that in a heterogeneous T cell population an additional production term is not required to explain the observation, instead,
the high rate of loss might only reflect loss from a small subset of rapidly dividing but short-lived T cells that are disproportionately labelled and the average rate of loss across the entire T cell population may equal the observed rate of production.

Interestingly, further investigation of T cell subpopulations has revealed that although the estimated rate of production exceeds the rate of loss in the memory CD4+ T cell compartment, the reverse is in fact true for naive T cells; recently divided naive T cells appear to have a survival advantage over more established naive T cells [62, 64]. More recently, mathematical models that explicitly account for heterogeneous cell populations have been developed and may provide further insight into the kinetic diversity that exists within the T cell population [65]. Although it remains difficult to determine the true rate of T cell loss with in vivo labelling data, the method has allowed researchers to estimate differential rates of production within different human T cell subsets; CD4+ T_{em} are observed to have the highest average rate of division of 4.7% of cells per day compared to 1.5% of CD4+ T_{cm} per day, and a rate of only 0.2% of naive CD4+ T cells per day [64].

An alternative approach to measuring cell division involves the use of cell cycle antigen Ki67. Ki67 is expressed in late stage G1, S, G2 and M of cell cycle and peripheral T cells can be stained, and counted, to quantify the proportion of cells that are not at rest at any given time. The rate of division that is inferred from Ki67 data is inversely correlated to the duration of Ki67 expression. A length that is likely to be related to cell cycle time, a measure that has proved surprisingly difficult to estimate in vivo. In vitro estimates for the inter-division time of T cells range from ~12 hours [66] to ~19 hours in CD4+ T cells [67], while in vivo studies of CD8+ T cells in mice suggest that the average division time is closer to ~8 hours [68]. In addition, there is some evidence to suggest that cells may accumulate in the G1 phase of cell cycle in HIV-infected individuals, suggesting that in this scenario Ki67 may overstate the production of cells through division [69]. Despite the difficulties associated with using Ki67 to explicitly estimate a rate of production, the marker is widely used to com-
pare rates of cell division in different cell populations in healthy individuals. In accord with in vivo labelling studies, \( \sim 0.5\% \) of naive CD4\(^+\) T cells and \( \sim 2\% \) of memory CD4\(^+\) T cells express Ki67 in healthy young adults [1]. Furthermore, the proportion of dividing naive and memory CD4\(^+\) T cells is seen to decline continuously from birth to \( \sim 20 \) years but appears to be stable in adults until the age of 70 years at which stage the rate of proliferation appears to almost double [1,2,70].

**Homeostatic mechanisms**

In this section the mechanisms that drive T cell homeostasis and turnover are discussed. T cell homeostasis has largely been studied in the context of lymphopenia and often in TCR transgenic mice which means that interpretation of the results can be difficult since T cell clones are often present at non-physiological frequencies. Furthermore, the study of T cell homeostasis is confounded by the fact that in addition to variation in the signals that are available to peripheral T cells, the ability of a T cell to respond to homeostatic cues may change with its age and situation. Despite this, murine studies have enabled the identification of several key components of homeostasis.

**Naive T cell homeostasis**

There appear to be at least two independent mechanisms involved in naive CD4\(^+\) T cell homeostasis; cytokine and self-peptide-MHC-TCR stimulation (as reviewed by Surh & Sprent [71]). Foreign antigen-induced activation typically leads to a rapid expansion of specific naive T cells and the production of a large number of effector cells, a small fraction of which will survive and attain memory phenotype. The role that this process plays in the long-term development of the naive T cell population is not well understood, although, it is generally thought not to have a major influence on naive T cell homeostasis since antigen-activated cells rapidly differentiate away from the naive phenotype. However, a number of studies have shown that TCR interactions with self peptide-MHC ligands are necessary for the maintenance of perip-
eral naive CD4+ T cells [72,73]. It is not known whether cells undergoing self-peptide MHC-induced homeostatic proliferation rely on distinct signals to cells undergoing antigen-MHC-induced proliferation. T cells undergoing homeostatic proliferation have been shown to express a subset of genes turned up by full antigenic stimulation, including those involved in cell cycle but excluding those associated with effector activity, however, the degree of induction appears to be significantly lower, suggesting that the intensity of the T cell response is dependent on the level of stimulation [74]. Mathematical modelling of lymphopenia-induced division in response to self-peptide MHC stimulation suggests that it is best described by a process whereby cells undergo independent, single, stochastic divisions, as opposed to a pre-programmed sequence of divisions that is thought to be induced in response to antigenic stimulation [68]. Furthermore, studies suggest that TCR-driven survival and proliferation are induced through two distinct pathways [75].

In addition to TCR-mediated signalling, it is well established that naive T cell homeostasis relies on cytokine signalling; removal of the $\gamma_c$ chain through which many common cytokines signal (including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21) results in the loss of the peripheral naive T cell population [76,77]. In particular, IL-7 is thought to play an essential role in proliferation and survival; in the absence of IL-7, naive T cells fail to divide and are short-lived [78–81]. Moreover, it has been shown that the basal levels of free IL-7 controls the size of the naive T cell pool [79]. The T cell response to IL-7 is thought to be tightly modulated by the IL-7R$\alpha$ chain (CD127) that dimerises with the $\gamma_c$ chain to form the IL-7 receptor [82, 83]. IL-7R$\alpha$ has been shown to be down-regulated in activated T cells [78]. Naive T cells have also been found to downregulate CD127 after contact with IL-7 but upregulate CD127 in the absence of IL-7 [84], suggesting a feedback mechanism through which T cells are able to respond to the concentration of IL-7 availability.

Murine studies have shown that both lck-mediated TCR and IL-7R signalling can induce naive T cell division in lymphopenic hosts [75,85], however, the relative contribution of the two mechanisms remains unclear. It is thought to vary
for specific T cell clones and depend on the severity of lymphopenia. The role of IL-7 may also vary with age; naive CD4$^+$ T cells derived from cord blood, but not adult naive CD4$^+$ T cells, are able to proliferate in response to IL-7 stimulation in the absence of TCR activation [86]. More recently, the same difference was observed between adult and neonatal murine recent thymic emigrants and stimulation was associated with faster down-regulation of IL-7R$\alpha$ in neonatal T cells [87].

**Memory T cell homestasis**

It is generally agreed that memory T cells are derived from naive T cells, however, the transition from naive to memory is not fully understood and is a long way from being quantified. The size of the memory pool remains fairly fixed in adults despite continuous antigen-induced expansion and the creation of new memory cells suggesting that some form of replacement takes place. A common paradigm has been that the expansion of a particular T cell clone will inevitably lead to the shrinking of other memory T cell clones and sequential heterologous viral infections have been observed to cause severe attrition of pre-existing lymphocytic choriomeningitis virus (LCMV)-epitope-specific CD8$^+$ memory T cells [88]. However a recent study showed that, although the size of the CD8$^+$ T$_{CM}$ population is tightly regulated, the T$_{EM}$ pool appears to be able to expand following repeated immunization [89]. Moreover studies of human cytomegalovirus infection did not detect any quantitative impact of infection upon numbers of blood-born influenza- and EBV-specific CD8$^+$ memory T cells [90], suggesting a complex relationship between the survival prospects of an individual T cell clone and the stability of the memory T cell pool as a whole.

In addition to the acquisition of new cells through differentiation, the memory T cell population is maintained by homeostatic proliferation; memory T cells are observed to divide more vigorously than naive T cells in both healthy children and adults [61]. Furthermore, *in-vivo* isotope labelling studies show that the CD4$^+$ T$_{EM}$ pool is turning over at a higher rate than the T$_{CM}$ pool [64].
Interestingly, transfer of CFSE-labelled cells and BrdU labelling revealed that the reverse is true of CD8$^+$ T cells; T$_{CM}$ cell homeostatic division was observed to outstrip T$_{EM}$ cell division in the CD8$^+$ pool [44].

Homeostatic proliferation of both CD4$^+$ and CD8$^+$ memory T cells is thought to require IL-7 and IL-15 [76, 91, 92]. Unlike naive T cell homeostasis, memory T cell survival and turnover appears to be largely independent of MHC-interactions in both CD4$^+$ and CD8$^+$ memory T cells [93–95]. CD8$^+$ T cells are able to sustain homeostatic renewal and function in the absence of MHC I [94] and although ablation of TCR signalling capacity in mature CD4$^+$ T cells results in impaired function and turnover, population size remains constant [96–98].

In order to maintain a stable memory T cell population, the addition of new cells through proliferation and differentiation must be countered by cell death. Mathematical modelling has been used to show that Fas-mediated apoptosis within the proliferating compartment can explain density-dependent survival of memory T cells in the absence of competition for resources [99]. The balance of anti-apoptotic (Bcl-2) to pro-apoptotic Bcl-2 family members appears to be another way in which T cell death is regulated. It has been shown that Bcl-2, a factor that blocks the induction of activation-induced cell death (AICD), is up-regulated following TCR-mediated activation. BIM (Bcl-2 interacting mediator of cell death) is an example of a pro-apoptotic factor that is simultaneously up-regulated following TCR triggering and cytokine deprivation. BIM is thought to induce cell death by binding Bcl-2 and abrogating the inhibition of cell death by Bcl-2 [As reviewed by Krammer et al. [100]]. Interestingly, CD8$^+$ T$_{EM}$ cells were associated with reduced resistance to AICD and decreased expression of anti-apoptotic factor Bcl-2 than T$_{CM}$ cells [101]. It appears as though the regulation of T cell death is finely tuned by the balance of complex molecular interactions that decide whether a cell lives or dies according to different conditions that may differ in different cells.
1.4 Age associated changes

In line with the normal physiological development of other organs and tissues, the immune system grows until adulthood and then maintains a relatively consistent size until old age. Studies have shown that the concentration of CD4$^+$ T cells (measured per microlitre of blood) decline exponentially with age from birth to adolescence [102, 103]; however, when allowances are made for the growth of blood volume with age, it can been shown that healthy children undergo an age-related increase in both total body naive and memory CD4$^+$ T cells [2]. At birth, the naive compartment constitutes over 90% of both CD4$^+$ and CD8$^+$ T cells [102, 103]. Aging coincides with a relative decline in naive T cell numbers; the ratio of naive to memory T cells approaches approximately 1:1 by age 20 years. This reflects the fact that the memory CD4$^+$ T cell population outgrows the naive CD4$^+$ T cell population from birth to early adulthood [2, 103]. Both the naive and memory T cell compartments remains relatively stable from age 20 years to old age [70, 102–104].

The T cell response is highly dependent on the ability of a T cell to recognise antigen peptides through the T cell receptor and the immense receptor diversity of the naive T cell population allows the immune system to respond to new antigens. Naive T cells express $\sim 10^7$ different TCR $\beta$-chains, each of which is paired with 100 different $\alpha$-chains, resulting in $\sim 10^9$ distinct T cell receptors [105,106]. In contrast, the receptor diversity of the memory T cell compartment is more contracted and consists of $\sim 10^5$-$10^6$ different TCR $\beta$-chains, each paired with one or two different $\alpha$-chains.

PCR/oligonucleotide hybridization studies have shown that the average frequency of TCR sequences (specific clones) within the naive CD4$^+$ T cell population are fairly constant between the ages of 20 and 65 years, however, the frequency of specific clones was found to be significantly higher in subjects aged 75-80 years [70]. This suggests that TCR diversity is well maintained in adults to age 65 years despite a decline in thymic production of new naive T cells. A similar pattern of TCR diversity was observed in the memory CD4$^+$
T cell compartment, although the repertoire of TCR-β-chains in the memory T cell compartment was shown to be \(\sim 10\)-fold less than that in the naive T cell compartment. In agreement with this, CDR3-Vβ spectratyping has been used to demonstrate the relative preservation of clonality in both CD31\(^+\) and CD31\(^-\) naive T cell subsets to age 60 years [56].

**Thymectomy as a model for premature aging**

Thymectomy is tolerated without clinical consequences in humans as young as 6 months old [107], whereas, early neonatal thymectomy in mice is known to inhibit the development of the peripheral T cell population [4]. Thymectomy is typically characterised by an initial drop in both CD4\(^+\) and CD8\(^+\) T cell numbers and TRECs [108–111]. Integral to the post-thymectomy dynamics is the fall in the size of the naive T cell compartment, while memory T cell number have actually been observed to increase [112–114].

Long-term studies in young adults who were thymectomised during early childhood reveal persistently low CD4\(^+\) and CD8\(^+\) T cell counts and smaller naive T cell compartments than healthy controls [113, 114]. However, thymectomised subjects are able to maintain a reasonable number of T cells and one explanation for this is the observed increase in peripheral division, as measured by Ki67 expression, among CD4\(^+\) naive T cells [114]. Interestingly, almost two decades after thymectomy \(\sim 50\%\) of naive CD4\(^+\) T cells remained CD31\(^+\) compared to \(\sim 70\%\) in healthy age-matched controls [113].

Despite their young age and lack of clinical symptoms, long-term follow-up studies have shown that the distribution of T cell subpopulations in thymectomised subjects resembles that of older individuals [114], suggesting that thymectomy may prove to be a useful model of premature aging. In support of this, a recent vaccine study reported that thymectomised children produced delayed antibody responses to tick-borne encephalitis vaccination that is more typical of elderly individuals [115], although, previous studies have shown no significant difference in antibody production to tetanus toxoid, measles and mumps [109, 116].
1.5 Mathematical Modelling in Immunology

Modelling in general can be used to both predict and explain the behaviour of the immune system. Most models will seek to simplify complex interactions between different components of the immune system in order to capture key relationships between different variables. For example, it would be almost impossible to model the behaviour of each individual T cell trafficking between the lymph nodes, spleen and blood. Instead, models of T cell population dynamics most often ignore spatial variations in T cell behaviour within a host in order to describe more general rates of cell production and loss across the entire T cell pool. Mathematics provides a language for expressing such models and a framework for analysing the consequences of the relationships and assumptions that are defined by the modeller.

Ordinary differential equation models

Ordinary differential equation (ODE) models are one of the simplest tools used in mathematical modelling. An equation is used to relate the value of a variable, in this case T cell population size, and its derivatives, which in the straightforward first order case will be the rate at which T cell numbers change with time. While only a small subset of ODEs can be solved analytically, computational/numerical methods allow solutions to be determined to a high degree of accuracy in most cases. The are many examples where ODEs are used in immunology.

One important use is in the quantitative interpretation of data from in vivo DNA labelling studies [58, 59, 61]. Typical models assume that T cells divide at some constant rate, $\rho$, and that labelled, or equivalently recently-divided cells, are lost at some constant rate, $d$, over the labelling and de-labelling period. This is modelled by a system of first order differential equations. The model is used to estimate the rate of division and death of T lymphocytes that best describes the experimental observations.

ODE models have also been used to study the within-host dynamics of par-
asites and diseases such as HIV [117, 118]. Perelson et al. [117] modelled the behaviour of uninfected T cells, infected T cells and free virus in HIV infection to great effect and were able to quantify the half-life of plasma virions as well as explaining the dynamics of early phase infection. The longer asymptomatic phase of HIV infection has been more difficult to explain. An ODE model was used by Yates et al. [119] to show that the chronic activation hypothesis, as it stands, in which elevated proliferation drives further virus production, can not explain the observed slow decline in CD4\(^+\) T cell numbers.

**Stochastic models**

A deterministic model is one in which a given starting point will always lead to the same outcome for a given set of parameters, such as rates of production and loss. In contrast, a stochastic model allows for some randomness within the parameters. For example, instead of a fixed value for the rate of division, \(\rho\), the rate might be defined by a probability distribution, say a normal distribution with mean \(\rho_\mu\) and standard deviation \(\rho_\sigma\). As a result, each model simulation will use a slightly different rate of division, chosen randomly from the distribution, and give a slightly different outcome.

In theoretical immunology, stochastic models are commonly used to interpret cell division data from carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling studies [66, 120–123]. CFSE fluorescence decreases approximately two-fold upon division and allows the division history of labelled cells to be tracked over time. The process of entering cell division or dying is thought to have a strong stochastic element which makes it ideally suited to be described by a probability distribution rather than a single rate. Studies have shown that the variation in time to first division by resting lymphocytes, *in vitro* at least, broadly conforms to a lognormal distribution [120]. Branching processes provide a useful way of modelling the random variation that exists at a single cell level as well as the changes that occur between division classes; in a typical model, the probability of undergoing the \(n\)th division is drawn from the same distribution for all cells that have divided \(n-1\) times.
Optimisation algorithms are used to identify the underlying parameters and standard probability distributions that best describe the observed CFSE data. This approach allows for the estimation of time to division, cell cycle time, and average death rate. However, a key limitation of this approach is that it is not always possible to identify a unique solution for underlying parameters of lymphocyte loss and division, particularly if the probability of division or loss changes with division number. An alternative approach has been to use mathematical modelling to differentiate between different models of cell division [68]. In this case a likelihood-method is used to identify the most feasible from two or more distinct theoretical models of cell division and loss.

**Age structured models**

Age structured models allow individuals, or lymphocytes, to be tracked according to their age and can provide deeper insight into the population dynamics. In this type of model, cells enter the youngest age class and then either progress naturally into an older class or die with some probability (which might be defined by a deterministic rate or a probability distribution). Age structured models allow the modeller to explore how the composition of the population might change with time, in addition to broader changes to total numbers. Models of this type are commonly applied in epidemiology to account for increased susceptibility to infection in elderly or very young individuals but can also be applied at a cellular level if there exists variation in the dynamic or functional properties of cells according to their age.

**Spatial models**

The development of spatial models of T cell dynamics have been enabled by advances in two-photon microscopy [24]. The imaging of T cell migration through intact lymph nodes, in combination with mathematical analysis, has led to the construction of spatially explicit models of DC and T cell migration patterns [124]. These models allow the rate at which T cells scan the lymph node environment to be estimated and provide insight into whether T cell mi-
Monte Carlo methods

Monte Carlo methods provide a powerful tool for solving mathematical problems that might otherwise be unfeasible analytically. The principle behind the Monte Carlo approach is to identify the probability distribution of some variable by repeating an experiment (simulation) to obtain observed values of the variable. If the simulations are repeated a sufficient number of times, it is assumed that the distribution of observed values will converge to the actual distribution. The method can be used to estimate the uncertainty (or variation) associated with some new variable that is a complex function of observable variables, such as age and weight, where the probability distribution for these variables is known.

The strength of the approach lies in its application to the solving of inverse problems and parameter estimation. The basic idea is to move randomly through the feasible parameter space and consider the probability of observing the experimental data for each parameter value that is chosen. The algorithm, known as the Metropolis algorithm [125], is more efficient than traditional methods since each sampled parameter value is accepted or rejected according to its likelihood.

1.6 Aims of this thesis

Aging is thought to be associated with a continuous decline in thymic production of mature naive T cells [8], although, the exact rate of involution and quantity of thymic output remain unknown. Naive T cells are thought to be relatively long-lived and relatively quiescent, however, human in vivo labelling studies suggest that low-level division and replacement occur continuously within the peripheral population. At the same time Ki67 expression suggests that there is a decline in the rate of peripheral expansion with age. The underlying mechanisms that allow the immune system to adapt to a decline in thymic
production while maintaining T cell diversity and numbers are not well understood.

The aim of this thesis is to better understand the role of T cell homeostasis in the development and maintenance of the naive CD4$^+$ T cell compartment in humans. The contribution of thymic production, peripheral expansion and survival to the development of the naive CD4$^+$ T cell population during childhood is investigated. The ability of the naive T cell population to persist following complete thymectomy is a testament to the effectiveness of T cell homeostasis. Mathematical modelling and experimental data are combined to explore the kinetics of the naive CD4$^+$ T cell compartment in normal aging and following thymectomy. The objective is to identify rules that determine T cell behaviour and might allow us to better understand the processes that drive T cell turnover and allow for the growth and maintenance of an effective naive T cell pool.
Chapter 2

Quantifying the development of the mature naive CD4$^+$ T cell pool in humans
Abstract

What are the rules that govern a naive T cell’s prospects for survival or division following export from the thymus into the periphery? In this chapter, experimental data is combined with robust mathematical models to estimate the absolute contributions of thymic export, division and loss to the population dynamics of the human naive CD4+ T cell pools between the ages of 0 and 20 years. Despite the observed decline in the frequency of these cells in blood with age, total body numbers of naive CD4 T cells are found to increase throughout childhood and early adulthood. Post-thymic proliferation makes a 2-fold larger daily contribution to total numbers than thymic export, and the relative contribution of thymic export and peripheral expansion appears to be constant until age 20 years. Our analysis suggests that as thymic export declines with age, healthy naive CD4 T cell turnover becomes progressively less dynamic; the average time between cell divisions lengthens and cell survival times increase. Together with other studies, our results suggest a complex picture of naive T cell homeostasis in which population size, time since export from the thymus or time since the last division can influence a cell’s prospects for survival or further divisions.
2.1 Introduction

An adult human has a population of roughly $10^{11}$ naive CD4$^+$ T cells circulating in their peripheral lymphoid organs and blood. From early in development this population is generated and sustained by thymic export and division in the periphery, and is estimated to comprise at least $10^8$ different T cell receptor specificities [105] providing a broad spectrum of protection in a diverse pathogen environment.

The rate of export of naive T cells from the thymus declines substantially with age in healthy individuals [8], but estimates of the total number of cells exported from the thymus over an individual’s lifetime are still approximately 10-fold greater than the total number of naive T cells in an adult at any one time\(^1\). Further, at least a subset of naive cells continues to divide slowly after release from the thymus into the periphery. These two observations imply that turnover and replacement occurs in the naive T cell pool. What are the rules that govern a circulating naive cell’s prospects for survival and proliferation? Do these rules change as we age, and if so, how? Identifying these rules is essential for understanding the dynamics of the T cell pool when dysregulated – for example, during the reconstitution of the T cell pools after medical interventions that induce lymphopenia, or following antiretroviral therapy in HIV infection. Addressing these questions requires a combination of experimental approaches and mathematical models.

As a first step, here the relative contributions of proliferation, loss and thymic input to the development of the healthy naive T cell compartment is quantified. The youngest age groups might be expected to have the most dynamic T cell populations, because rates of thymic export are highest and physiological growth, in particular, growth of blood volume and lymphoid tissue, is continually altering the environment in which the T cells circulate and encounter homeostatic signals.

\(^1\)The estimate of daily thymic output by [8] in equation (2.3) integrated over 80 years gives $5 \times 10^{12}$ cells.
Currently the most direct methods for measuring lymphocyte dynamics \textit{in vivo} employ deuterated glucose or heavy water to label dividing cells. Interpretation of some of the data from these studies is controversial, however, and for ethical reasons the approach is difficult to use in children. Much of our understanding of the population dynamics of the human naive T cell pool from early in development to young adulthood has been gained through more indirect methods and through extrapolation from mouse studies. Most commonly, the contribution of thymic export has been assessed using the frequency of T cells in blood expressing markers of recent exit from the thymus and the T cell receptor excision circle (TREC) content of naive cells. TRECs are circular nuclear DNA fragments that are stable remnants of the recombination events that generate T cell receptor diversity in the thymus, and are shared randomly between daughter cells upon division. The mean per-cell TREC content of the naive T cell pool is a poor measure of thymic function, however, since TRECs are diluted by division in the periphery. This is particularly problematic when trying to compare thymic function across different levels of lymphopenia, since rates of peripheral division may vary substantially. In contrast, total TREC numbers are not affected by peripheral division. Measurement of total TRECs in the naive pool, rather than TREC frequency, thus provides (somewhat noisy) information regarding the balance between thymic export and the removal of naive cells through loss, cell death or differentiation [54].

Naive CD4$^+$ T cells are maintained homeostatically in the periphery by competition for cytokines and possibly self-peptide/MHC signals [92]. Average rates of division and/or loss are then expected to depend on the total number or density of cells competing for these resources. Evidence for such competition or resource-limitation comes from studies in which lymphopenia and the availability of survival or proliferative stimuli are manipulated in mice, resulting in changes in the survival times and rates of homeostatic proliferation of naive T cells [68,79]. Previous approaches to modeling homeostasis of replete T cell pools have represented the notion of competition or quorum-sensing with heuristic forms for the average rates of proliferation and loss as functions of
total population size [2, 99, 126, 127]. However, the predictions and insights gained from these models may then be contingent on the specific forms of these functions.

In this work, data from a number of existing human studies is combined with a simple and quite general mathematical model of naive T cell population dynamics. This approach allows us to estimate the absolute contributions of thymic export, proliferation and loss to total body naive CD4$^+$ T cell numbers, and how these contributions change over time between birth and young adulthood. Importantly, this model makes no assumptions regarding the density dependence of proliferation and loss of naive cells in the periphery, and so provides robust estimates of the age-dependent rates of these fundamental processes in humans.

2.2 Mathematical model

Estimating total body naive CD4$^+$ T cell numbers

In order to measure the contributions of division and loss to the naive pool, estimates of total naive cell numbers as a function of age are required. Peripheral naive T cell levels are typically quoted in units of cells per ml or µl of blood, and these values decline with age [102, 103]. However, as noted previously by Hazenberg et al. [2], the additional information of blood volume, which increases during early life, is needed to estimate total body naive T cell numbers. Further, naive T cells circulate continuously between blood and lymphoid organs and approximately 2% are found in the blood at any moment [23]. In what follows, then, total body naive T cell numbers is estimated by multiplying the cells per unit volume of blood by 50 × total blood volume.

To our knowledge, there are no published studies directly specifying the dependence of mean blood volume on age. Instead published estimates of the dependence of blood volume on body weight (Figure 2.1B), and of mean body weight on age are used (Figure 2.1A). These are brought together with an
2.2 Mathematical model

estimate of the age-dependence of naive cell density per microlitre of blood in healthy children (Figure 2.1C) to give a non-linear function for mean total body naive cell numbers as a function of age (Figure 2.1D). A Monte Carlo approach is used to combine the uncertainties in these regression estimates and the population-level variations in physiology to estimate the distribution of total naive T cell pool sizes across individuals of a given age. Details of this procedure can be found in Appendix 2.5.1.

There is some evidence that the proportion of cells in blood itself may be age-dependent; in Appendix 2.5.2 it is shown that this has only a minor effect on our conclusions.

Figure 2.1: A: Body weight as a function of age (from Kuczmarski et al. [128]). B: Blood volume as a function of age, estimated from panel A and the regression relation between weight and blood volume in Linderkamp et al. [129]. C: Mean naive CD4$^+$ T cell density in blood with age, using the relation in Huenecke et al. [103]. D: Estimated total body naive CD4$^+$ T cell numbers, using the data in panels B and C. Panels A, B, C and D show the mean and estimated 95 percentiles at the population level.
2.2 Mathematical model

Estimating the distribution of total body naive T cell TRECs

Douek & colleagues [1] measured the frequencies of TCR rearrangement excision circles in naive CD4$^+$ T cells (as TRECs per $\mu$g of DNA) in healthy individuals aged 0 to 80. Using their data for individuals aged 0 to 20 years only, no significant change in the TREC content of naive CD4$^+$ T cells was found with age, although there was considerable variation between individuals (Table 2.1; Figure 2.2A; p=0.11). Stability of TRECs during childhood is also observed in other studies of naive CD4$^+$ T cells by Douek et al. [52], naive CD8$^+$ T cells by McFarland et al. [130], and PBMCs by Zhang et al. [131]. Assuming that 1 $\mu$g of DNA represents 150,000 cells, the mean TREC number ($\tau$) per naive CD4$^+$ T cell in this age range is estimated to be 0.084 ($\pm$0.01 s.e.m.), and the values of $\tau$ among the pooled individuals were well described by a lognormal distribution with parameters, $\mu$=-2.4 and $\sigma$=0.46. Again using a Monte Carlo approach, this distribution is combined with our empirical distribution of total naive T cell numbers to to generate an empirical distribution for total body TRECs ($= \tau N(t)$) across the population as a function of age (Figure 2.2B).

<table>
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<th>Age (years)</th>
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Table 2.1: Observed frequency of TRECs per naive CD4$^+$ T cell in healthy individuals age 0 to 20 years. Data from Douek et al. [1]
2.2 Mathematical model

Figure 2.2: **A**: Measurements of TRECs per naive CD4$^+$ T cells, taken from [1]. **B** Estimated distribution of total TRECs in the naive CD4$^+$ T cell pool.

**Naive CD4$^+$ T cell and TREC dynamics**

A very general population model is used to explore the dynamics of naive T cells with an input term from thymic export, a term for population growth (division in the periphery that preserves naive phenotype) and one for cell loss. As above, we let $N(t)$ be the total number of naive CD4$^+$ T cells in the body at age $t$, measured in days. Changes in this population with age $t$ are described using a generalised version of a standard model of naive cell dynamics used by several authors [53, 54, 126]:

$$\frac{dN(t)}{dt} = \theta(t) + \rho(t)N(t) - \delta(t)N(t),$$

where $\theta(t)$ is the rate of export of cells from the thymus (cells day$^{-1}$), $\rho(t)$ is the per-cell rate of addition to the naive pool through successful cell division (day$^{-1}$), and $\delta(t)$ is the per-cell rate of disappearance of cells from the naive pool (day$^{-1}$). The latter is a composite of cell death, change of phenotype and loss. From here on these processes are collectively referred to as ‘loss’.

In differential equation models of this sort, the per-cell ‘rate’ of a process is a measure of the probability any given cell will undergo that process in a short time interval. The quantity $1/\rho(t)$ can be interpreted as approximately the expected time to the next successful division of a naive cell in an individual of age $t$, and $1/\delta(t)$ is approximately the expected residence time of a CD4$^+$ T cell in the naive pool in an individual of age $t$. 
The mean rates of division and loss in the periphery are allowed to change with age, but in this model we do not impose functional forms for this age-dependence nor do we assume any particular dependence of these rates on the pool size $N(t)$. Note also that naive T cells of different specificities and recent thymic emigrant/mature naive cell status are treated as a homogeneous population; here it is the mean rates of division and loss across the whole naive CD4$^+$ T cell compartment that are of interest.

We let the total TREC s in the naive CD4$^+$ T cell pool at age $t$ be $T(t)$. $T$ is only influenced by thymic export and loss, but not division. The model first described by Hazenberg et al. [53] is used to describe TREC dynamics in the naive population:

$$\frac{dT(t)}{dt} = c \theta(t) - \delta(t)T(t), \quad (2.2)$$

where $c$ is the mean TREC content of cells emerging from the thymus, and $\theta(t)$ and $\delta(t)$ are defined above. It is assumed that intracellular degradation of TRECs over time is negligible, and that the average TREC concentration of CD4$^+$ T cells emerging from the thymus, $c$, is constant up to age 20 years [132]. Junge et al. [133] reported an average 250 TRECs per 1000 recent thymic emigrant (CD31$^+$) CD4$^+$ T cells at birth, giving an estimated lower bound of $c = 0.25$. Okamoto et al. [134] measured the TREC content of single-positive CD4$^+$ thymocytes in neonates to be $\sim 0.6$; this provides a conservative upper bound for $c$ since cells are expected to undergo division between acquisition of single-positive status and exit from the thymus. In what follows, let estimate $c=0.25$.

**Modeling thymic export**

Thymic function is assumed to be proportional to the volume of the thymic epithelial space (TES). Steinmann et al. [8] showed that the involution of the TES with age could be described well by a modified exponential decay function. There remains an unknown constant of proportionality that relates the the volume of the TES to the rate of naive CD4$^+$ T cell export from the thymus in cells/day. The proportionality constant is estimated by scaling the Steinman
2.3 Results

decay function to obtain a daily thymic export rate of $10^8$ cells per day at age 30, in accord with previous studies [126,135]. This is a combined predicted output of both CD4 and CD8 T cells. Jamieson et al. [132] showed that the ratio of CD4 to CD8 single positive mature thymocytes in fetal and adult thymi were similar, and the average CD4 proportion was observed to be 0.68. This gives an estimated thymic output of $6.8 \times 10^7$ naive CD4$^+$ T cells per day at age 30. This is used to calibrate the thymic export function and derive the following estimate for the mean rate of thymic export as a function of age $t$:

$$\theta(t) = 2.32 \times 10^8 \exp(-1.1 \times 10^{-4}t) + 1.15 \times 10^8 \exp(-1.6 \times 10^{-7}t^2).$$ (2.3)

Applying this estimate of thymic export to equations (2.1) and (2.2), rates of cell division and loss ($\rho(t)$ and $\delta(t)$) can be estimated without making any further assumptions, by numerically estimating the rate of change of total cells $dN/dt$. That is, the rate of growth of the total body naive pool is calculated using the calculated total naive T cell numbers with age (Figure 2.1D). Where the constant factor used to calibrate thymic output function directly scales our estimates of the absolute values of peripheral production and loss in the periphery.

2.3 Results

The interdivision time of naive CD4$^+$ T cells increases with an individual’s age

If TREC$s$ per naive cell are constant, as suggested by the data from Douek et al. [1], the rate of cell division, $\rho(t)$, becomes proportional to thymic export and inversely proportional to the size of the naive pool (see Appendix 2.5.3):

$$\rho(t) = \left(\frac{c}{\tau} - 1\right) \frac{\theta(t)}{N(t)}. \quad (2.4)$$

Hence, to maintain the observed constant TREC frequency $\tau$, the rate of suc-
cessful cell division in the periphery must decrease as thymic export wanes with age. $\rho(t)$ is numerically estimated from equation (2.4) using the estimated thymic export function $\theta(t)$ as defined in equation (2.3) and observed naive T cell numbers (Figure 2.1D). The mean interdivision time for naive cells, $1/\rho$, is found to be approximately 125 days for children between the ages 1 and 5 years, or that approximately 0.8% of the population is dividing on any given day. By age 20 years, the interdivision time is estimated to increase to approximately 500 days (Figure 2.3A), corresponding to 0.2% of the population dividing per day. This is comparable with a rate of naive T cell production of 0.2% per day obtained from an in-vivo deuterated glucose labeling study in young adults [64]. However, in vivo labeling studies are unable to distinguish between cells that have recently divided in the thymus and post-thymic proliferation. The observed rate of division from such studies is a composite of both these processes and represents an absolute upper bound on $\rho$.

Figure 2.3: Estimated mean rate (solid black line) rate of successful naive CD4$^+$ T cell division, $\rho(t)$ (panel A), and the mean rate of loss, due to death, change of phenotype and migration, $\delta(t)$ (panel B) as functions of age $t$ (assuming thymic output of $6.8 \times 10^7$ CD4 cells per day at age 30.) Panels show mean and estimated 95th percentiles.

The expected residence or survival time of naive CD4$^+$ T cells increases with age

The expected rate of loss of naive T cells, is calculated from equation (2.2) and the relation $T(t) = \tau N(t)$:
The expected residence time ($1/\delta$) of a naive CD4$^+$ T cell in the periphery is of the order of 70 days between the ages 1-5 years, increasing to approximately 400 days by age 20 years, corresponding to a rate of death of 1.4% and 0.3% of the population per day respectively (seen in Figure 2.3B). Assuming that the system is in a quasi steady-state over short-time scales, i.e. homeostasis, the rate of loss is approximately equal to the rate of production (peripheral division and thymic production). This is referred to as the rate of turnover. Our estimated rate of turnover in young adults, $\delta \approx 0.003$ day$^{-1}$, is in accord with the turnover rate estimated in the deuterated glucose labeling study of Macallan et al. [64] but is 5-fold higher than the estimate obtained by Vrisekoop et al using heavy water [62]. The discrepancy between these studies is unresolved. However, our estimates of the absolute rates of division and loss are directly dependent on the estimated thymic export function, and scale linearly with the constant of proportionality used to calibrate this function. Our conclusion that both rates decline with age is independent of this scaling constant, however.

**Between ages 0-20, the absolute daily contribution to the naive CD4$^+$ T cell population from peripheral division is greater than the contribution from thymic export**

From equation (2.4) and using the estimates $c \geq 0.25$ and $\tau \approx 0.08$, the total number of cells produced by successful cell division in the periphery each day
2.4 Discussion

The ratio $c/\tau$ is a measure of the TREC dilution that occurs due to proliferation in the periphery. Equation (2.6) predicts that the daily contribution of peripheral division to the pool will necessarily be greater than that of thymic export whenever $c > 2\tau$. Experimental estimates of $c$ and $\tau$ imply that the contribution of cells to the naive pool through proliferation at any given age is at least 2-fold larger than the contribution from the thymus (Figure 2.4). Since both $c$ and $\tau$ appear to be constant to age 20 years, equation (2.6) tells us that the ratio of the total number of cells dividing per day to the total cells exported by the thymus per day remains approximately constant until age 20 years.

2.4 Discussion

Our analysis makes three points. First, it suggests that in humans, despite their decrease in frequency in the blood with age, total body numbers of naive CD4$^+$ T cells grow almost continuously from (before) birth well into early adulthood. Second, it shows that the mean interdivision time of these cells increases almost continuously with age. This is in keeping with the conclusions of Hazenberg et al. [2] who showed division rates falling between the ages of 0-5 years; our analysis predicts that this decline continues into adulthood. It has been reported elsewhere that rates of naive T cell division are very low in adults [64, 136]; however, this study suggests that in children naive proliferation is relatively vigorous, with an interdivision time of the order a few months. Third, it shows that the net rate of exit from the naive CD4$^+$ T cell pool (the combination of apoptosis, loss and differentiation) falls with age, or in other words the mean residency time of a naive T cell increases with an individual’s age. Since thymic export falls with age, together these results suggest that as the naive T cell pool matures it becomes progressively less dynamic.

It is usually assumed that thymic export makes the dominant contribution to the naive T cell population in children, but the study by Hazenberg et al. [2]
showed that peripheral division also provides a substantial fraction of the daily production of new cells. Our analysis confirms and quantifies this, up to age 20, at most 30% of the new naive cells that appear each day are produced in the thymus. Interestingly, the invariance of TREC frequencies in peripheral naive T cells suggests that this proportion remains fixed during early life; there is apparently no increase in rates of division in the periphery to compensate for the drop in thymic production. One potential explanation for this apparently stable relationship between the rate of thymic export and division is that recent thymic emigrants may divide more rapidly than mature naive cells.

**Density-dependent homeostasis?**

Dutilh et al. [126] showed naive T cell TREC dynamics can be explained by models of homeostasis in which rates of proliferation and/or loss are linked to the size of the naive population; that is, these rates are density-dependent. Such ecological models of the naive pool assume reasonably that as cell numbers increase, so should competition for resources and so rates of division fall and rates of cell death increase. However, our results show that both the rates of proliferation and loss (on a per-cell basis) fall with increasing pool size. This raises the question of whether naive T cell competition is sensitive to cell densities (e.g. cells per unit volume of blood), which fall with age, or to total body
numbers, which appear to increase with age. If proliferation is limited by competition for signals or resources such as IL-7, our analysis suggests that the likelihood of a cell dividing within a given time interval is limited by the naive population size rather than the naive cell density in blood. It has also been argued that naive CD4 populations may be regulated at the level of individual clone size rather than total cell numbers \[137\], perhaps through competition of cross-reactive TCRs for overlapping ‘niches’ of self-peptide MHCII complexes in the periphery.

The picture is less clear in the case of cell loss, since here (and in other models) the naive CD4 T cell loss rate \( \delta(t) \) is a combination of death, differentiation to effector or memory phenotypes, or permanent loss from circulation. Here the effects of these factors can not be isolated, and so it is not possible to assess how rates of apoptosis induced by homeostatic resource limitation depend (if at all) on cell numbers.

**Phenotypic heterogeneity and clonal diversity in the naive T cell pool**

Despite the slow increase in naive T cell numbers over the timescale of years, over short timescales (days or weeks) the pool is in a state of ‘quasi-homeostasis’, where the number of cells lost or displaced from the naive pool each day is approximately equal to thymic production and peripheral division combined. However, our model tells us nothing about whether this displacement occurs randomly or whether some naive subpopulations have a selective advantage. One strong possibility is that there may be heterogeneity in expected survival time with respect to the time since the last division. Vrisekoop et al. \[136\] performed isotope-labelling assays in healthy young adult volunteers and found that newly produced cells (i.e. cells that had taken up label, either through division in the thymus and export soon after or through division in the periphery) were lost at a very low rate – much lower than the rate of successful division. This implies that recently divided naive cells have a survival advantage over the average cell in the pool as a whole. Thus one would pre-
dict that as division rates fall with age, average loss rates would increase – not decrease, as our analysis indicates. To resolve this apparent inconsistency requires that the per-cell loss rate of naive T cells depends both on time since last division and also on an individual’s age, either explicitly or indirectly through (for example) population or clone size. This suggests a two-layered mechanism for maintaining clonal diversity in the naive T cell pool; the aim being (i) to provide recent thymic emigrants, which are costly to produce and help to increase our TCR repertoire, with a survival advantage and (ii) to increase naive cell longevity as thymic output wanes. Precisely how this regulation of cell loss is regulated with age, however, remains an open question.

Together these results suggest a more complex, structured picture of naive T cell dynamics than has perhaps previously been considered. Per-cell division rates may vary as a function of age, total naive T cell numbers or cell density, through resource competition. They may also vary as a function of time since thymic export. Further, survival may depend on the time since last division as well as resource availability. How then do the signals T cells receive from their environment translate into survival or proliferative benefits? Since IL-7 is required for both naive T cell survival and division, cells that have accumulated sufficient signal to divide may well have the additional benefit of a survival advantage through (for example) accumulation of anti-apoptotic factors. Interestingly, memory T cell populations appear to follow different rules; in the CD4⁺CD45RO⁺ population, recently-divided cells appear to be more prone to apoptosis than quiescent cells [61].

2.5 Appendices

2.5.1 Calculating the distribution of total body naive CD4⁺ T cells with age

Linderkamp et al. [129] reported the following linear relationship:

\[
\log (\text{blood volume (\(\mu\)l)}) = 0.97 \log (\text{body weight (kg)}) + 4.93, \quad (2.7)
\]
where the standard error on the slope is 0.05. Average body weight as a function of age is determined using standard growth charts for healthy children, published by the CDC [128]. Specifically, for fixed age \( t \), we repeat the following:

1. Draw the logarithm of body weight, \( \log W(t) \), from a normal distribution with mean \( \log(W_{50}(t)) \) and estimated standard deviation

\[
\sigma = \frac{1}{4} \log \left( \frac{W_{97}(t)}{W_{3}(t)} \right),
\]

where \( W_i(t) \) is the \( i \)-th percentile of body weight at age \( t \) [128] (Figure 2.1A);

2. Calculate a blood volume \( V(t) \) using the body weight \( \log W(t) \) generated in the previous step; \( V(t) \) is drawn from a lognormal distribution with mean given by equation (2.7) and standard deviation calculated from the standard error on the slope of the regression line (the distribution of total blood volume across individuals by age, obtained by combining steps 1 and 2, is shown in Figure 2.1B);

3. Calculate naive (CD45RA\(^+\)) CD4\(^+\) T cell density per microlitre of blood, \( \nu(t) \), from the fitted relationship calculated by [103] (Figure 2.1C):

\[
\nu(t) = 496 + 2074 \exp(-8.75 \times 10^{-4}t),
\]

with upper and lower 95% bounds defined as \( (\nu(t) - 0.1, 2\nu(t)) \). Draw values for \( \nu(t) \) from a lognormal distribution with parameters \( \mu = \log[\nu(t)] - 0.1 \) and \( \sigma = 0.45 \), chosen to fit a mean of \( \nu(t) \) and 95% interval as defined above.

4. Total body naive (CD45RA\(^+\)) CD4\(^+\) T cell numbers are then estimated from their blood density using \( N(t) = 50\nu(t)V(t) \), with the factor 50 accounting for the estimated 2% of naive cells in the blood at any time.
From the empirical distribution generated by repeating steps 1-4, the mean values for total body naive CD4$^+$ T cell numbers at age $t$, along with the estimated 2.5% and 97.5% quantiles are estimated. This procedure is then repeated for ages in the range 0-20 years (Figure 2.1D).

### 2.5.2 Modelling the age-dependent distribution of naive T cells between blood and secondary lymphoid organs

An important assumption of our study is that the proportion of the naive T cell pool found in the blood is independent of age. However, Watanabe et al. [138] show that the ratio of spleen volume to body weight declines slightly from the age of 0 to 20 years. If it is assumed that lymphoid tissues grow proportionally with the spleen, then their results suggest that percentage of lymphocytes found in the blood increases linearly from approximately 1% at birth to 2% at age 20 years.

When this is included in the analysis, a more rapid expansion of total body cell numbers is observed early in life, approaching a mean of $1.3 \times 10^{11}$ cells by the age of 2 (Figure 2.5). In terms of predicted rates of cell production and loss this translates into slower dynamics, however, we still expect to see a general decline in both rates with age, although the drop is less steep. In terms of absolute values, the predicted total number of cells produced each day does not change as this is simply a function of our estimated thymic function (equation 2.6). The total number of cells lost from the naive pool is marginally lower between the ages of 0 to 5 to allow a steeper rate of cell population growth at this younger age. Figure 2.5 D illustrates that although the size of the total naive pool is sensitive to the assumption about proportion of lymphocytes found in peripheral blood, the predicted rate of cell production and loss (cells/day) are fairly robust to changes in this assumption.
Figure 2.5: Measuring sensitivity to proportion of cells found in lymph nodes: **A:** Total body naive population as a function of age. **B:** Predicted rate of proliferation. **C:** Predicted rate of loss. **D:** Predicted absolute number of cells exported by thymus (solid line), produced in periphery (dashed) and lost from naive pool (dotted line). (Calculated assuming the proportion of cells in the blood increases linearly from 1% at birth to 2% at age 20 (dark line); fixed 2% for all ages (grey line))
2.5 Appendices

2.5.3 Modelling constant TREC densities

The observation that the TREC content per naive CD4 T cell, $T(t)/N(t)$, is a constant, $\tau$, implies

$$\frac{d}{dt} \left( \frac{T}{N} \right) = 0 \implies \frac{1}{N} \left( \frac{dT}{dt} - \frac{T}{N} \frac{dN}{dt} \right) = \frac{1}{N} \left( \frac{dT}{dt} - \tau \frac{dN}{dt} \right) = 0. \quad (2.8)$$

Equation (2.4) is obtained by substituting the expressions for $dN/dt$ and $dT/dt$ from equations (2.1) and (2.2) respectively.
Chapter 3

Quantifying Thymic Export: combining models of naive CD4$^+$ T cell proliferation and TREC dynamics gives an explicit measure of thymic output
Abstract

Understanding T-cell homeostasis requires knowledge of the export rate of new T-cells from the thymus; a rate that has been surprisingly difficult to estimate. In the previous chapter the relative contributions of peripheral post-thymic division and thymic export to the dynamics of the naive T cell pool were explored. In line with previous studies, it was assumed that thymic volume was directly correlated to thymic output and estimated the number of T cells exported per day by scaling a function fitted to thymic epithelial space (TES) volume with age. Here an alternative method for quantifying thymic export in humans on an individual basis, by combining two simple mathematical models, is presented. One model uses Ki67 data (from Douek et al. [1] and Hazenberg et al. [2]) to calculate the rate of peripheral naive T-cell production whilst the other tracks the dynamics of T cell receptor excision circles (TRECs) (data from Douek et al. [1]) . TREC content has been used as a proxy for thymic export, but this quantity is influenced by cell division and loss of naive T-cells and is not a direct measure of thymic export. Combining these models allows the contributions of the thymus and cell division to the daily production rate of T cells to be disentangled. The method is illustrated with published data on Ki67 expression and TRECs within naive CD4+ T cells in healthy individuals. A quantitative estimate for thymic export is obtained as a function of age from birth to 20 years. This analysis shows that the export rate of T cells from the thymus follows three distinct phases: an increase from birth to a peak at 1 year followed by rapid involution until around 8 years and then a more gradual decline until 20 years.
3.1 Introduction

The thymus is the primary source of naive T cells and plays a key role in establishing and maintaining the peripheral T cell pool. In children, the T cell compartment grows continuously with age from birth to adulthood. Cell numbers then remain approximately stable throughout adult life. During the first 20 years of life the thymus is known to involute and its output is supplemented by division within the existing peripheral naive T cell pool [1, 8, 53, 61]. However, the absolute contribution of the thymus to the peripheral naive cell pool and how this contribution changes with age is difficult to measure. Despite a wide array of immunological markers, imaging techniques and histological studies, we still lack a direct quantitative measure of thymic export and are unable to answer some basic questions about the contribution of the thymus to lymphocyte homeostasis in health and in disease.

In humans, much of our knowledge of the thymus comes from biopsy studies. Steinmann and colleagues [8] showed that the thymus reaches its maximum volume by 1 year of age and then remains constant, but the relative size of the intra-thymic compartments changes substantially with age. The thymic epithelial space (TES) involutes by 70% over the first 20 years accompanied by a simultaneous expansion of the perivascular space, connective and adipose tissue. As the TES is the main site of thymopoiesis and the majority of thymocytes are found in this region, it is inferred that the functionality of the thymus must involute continuously with age. In chapter 2, TES volume was scaled to predict changes in thymic export with age, in accord with other studies [126], however, it is not obvious that the volume of the TES is directly proportional to the total rate of export of T cells to the periphery. The TES is a site of extensive expansion and selection where less than 5% of cells survive the development process and any minor changes in the rate of production or loss of cells may have a large impact on the rate of export [5–7]. In fact, even the decline in cellular density of thymic tissue with age, does not appear to directly parallel the changes in TES [139].
In-vivo isotope labelling has been useful for quantifying lymphocyte turnover in the naive CD4$^+$ T cell population in adults [62, 64], but there are difficulties associated with using this approach to quantify thymic export and the rate of involution. The method does not allow cells dividing in the periphery to be distinguished from those that divided in the thymus and emigrated to the periphery during the period of label administration.

T cell receptor excision circles (TRECs), the cell surface marker CD31 and, more recently, protein tyrosine kinase 7 (PTK7) expression by peripheral T cells have been used as surrogate markers for thymic export. TRECs are stable, non-replicative extra-chromosomal circles of DNA excised during T cell receptor gene rearrangement [51, 52, 140]. TRECs are produced in the thymus and can be measured in peripheral CD4$^+$ and CD8$^+$ T cells. CD31 identifies a subset of naive cells which is significantly enriched with TRECs [55]. PTK7 is expressed at high levels on mature CD4$^+$ CD8$^-$ thymocytes and a subset of naive CD31$^+$ CD4$^+$ T cells which is highly enriched for TRECs [3]. Both the TREC content and the proportion of recent thymic emigrants within the naive pool, defined by CD31 or PTK7 expression, are known to decline with age [3, 52, 55, 56, 112, 133]. However, these observations reflect a combined consequence of changing thymic export, cell division, and longevity of thymic emigrants and cannot therefore give a strict measure of declining thymic export [53, 56, 126, 141–143].

Ki67 is a nuclear cell cycle marker that is expressed only in proliferating cells from late stage G1 and is rapidly degraded on exit from cycle [144, 145]. Ki67 expression coincides with the deterministic phase of cell cycle [146–148] and provides a good estimate for the fraction of dividing cells at any given time. The level of proliferation within the naive CD4$^+$ T cell pool as determined by Ki67 expression declines with age [1, 2] and this must be taken into account when using the surrogate markers above to infer changes in thymic function over time.

In this work, thymic export is investigated by considering expression of both Ki67 and TRECs within the naive CD4 T cell population. The concentration
of TRECs per naive cell is a function of both thymic export and peripheral cell division and in chapter 2 a mathematical model was used to identify a relationship between these two contributing factors. A model of Ki67 expression data is used to quantify the contribution of peripheral expansion and applied to the model of TREC concentration to extract a quantitative estimate for the rate of thymic export.

![Diagram of T cell dynamics](image)

Figure 3.1: A simple model of naive CD4$^+$ T cell dynamics. The naive population is divided into two compartments according to cell cycle status; resting or dividing, where Ki67 expression is used as a surrogate marker for dividing cells.

### 3.2 Mathematical model

A mathematical model is described, combining TREC dynamics with naive T cell turnover (in turn measured by Ki67 expression), to allow thymic export from birth to adulthood to be quantified. Ki67 expression provides an independent, quantitative estimate of post-thymic production of naive CD4$^+$ T cells through peripheral cell division. This estimate is then substituted into a model of TREC dynamics to obtain an explicit expression for thymic export in terms of number of CD4$^+$ T cells that are exported per day. Ki67 expression and TREC content have been described in the naive CD4$^+$ T cell population as it grows from birth to adulthood. These data are used with the model to estimate thymic output over the first 20 years of life.
Using TREC\s to determine the relative contributions of thymic export and post-thymic expansion

As in chapter 2, a model initially presented by Hazenberg et al. [53] is adapted to interpret the expression of TREC\s by simultaneously studying two pools; total body naive T cells and total body TREC\s. The total naive CD4$^+$ T cell population at age $t$, $N(t)$, is described by a general population growth model allowing for cells emigrating from the thymus, expansion through peripheral division and cell loss through death, change of phenotype or migration out of the peripheral pool. From here on 'loss' is taken to be a combined function of all the process that lead to loss of naive cells from the peripheral system. The growth of the naive T cell population with age is described as follows:

$$\frac{dN(t)}{dt} = \theta(t) + \rho(t)N(t) - \delta(t)N(t)$$ \hspace{1cm} (3.1)

where $\theta(t)$ represents the rate of thymic export (number of CD4$^+$ T cells exported per day in an individual of age $t$); $\rho(t)$ (day$^{-1}$) is a per-cell rate of addition to the naive population through peripheral division at age $t$, approximately the inverse of the mean interdivision time of a naive cell; and $\delta(t)$ (day$^{-1}$) is the average rate of naive cell loss at age $t$, where $1/\delta(t)$ is approximately the expected residence time of a cell in the peripheral naive pool.

The following assumptions are used to define the dynamics of total body naive TREC\s: (i) TREC\s are exclusively produced following TCR gene rearrangement within the thymus [52]; (ii) cell division does not result in the loss or creation of TREC\s [52]; and (iii) intracellular degradation of TREC\s is negligible [52, 132, 140]. These assumptions imply that the loss of naive TREC\s is exclusively associated with the loss of naive T cells. Further, it is assumed that the rate of loss is homogeneous with respect to TREC content and that the average rate of naive CD4$^+$ T cell loss is equal to the average rate of naive TREC loss, as described by other investigators [2]. Changes in total naive TREC\s with
age can then be described by

\[
\frac{dT(t)}{dt} = c(t) \theta(t) - \delta(t) T(t),
\]

(3.2)

where \(T(t)\) represents the total number of TRECs in an individual age \(t\), \(c(t)\) is the average TREC content of CD4\(^+\) T cells emerging from the thymus, and \(\theta(t)\) and \(\delta(t)\) are defined above.

Jamieson et al. [132] showed that the TREC content of thymocytes remains constant with age, however, this is found not to be the best quantitative estimate for TREC content of cells emigrating from the thymus as it is measured in unsorted thymocytes and includes cells that have yet to undergo T cell receptor rearrangement and does not account for division within the thymus before export. Junge et al. [133] reported on average 250 sjTRECs per 1000 recent thymic emigrants (CD31\(^+\) CD4\(^+\) T cells) in cord blood. As discussed in chapter 2, this represents a lower bound on \(c\) as any post-thymic division results in dilution of TRECs. It has been shown that approximately 3 divisions occur within the thymus between the production of signal-joint and coding-joint TRECs [52]. Given that it is possible to produce at most 2 sjTRECs per cell if rearrangement occurs in both alleles, one can argue that a maximal value for \(c\) would be 0.25. In the following calculations it is estimated that \(c=0.25\).

TRECs are generally measured and reported as a number per \(\mu\)g of T cell DNA. This can be translated into an average number per cell using the assumption that 1\(\mu\)g of T cell DNA represents approximately 150,000 T cells. Define a new variable \(\tau(t) (= T(t)/N(t))\) to measure the average number of TRECs per naive CD4\(^+\) T cell. Equations (3.1) and (3.2) can be brought together to obtain an expression for rate at which \(\tau(t)\) changes with age (see appendix 3.5.1):

\[
\frac{d\tau(t)}{dt} = \frac{\theta(t)}{N(t)} (c - \tau(t)) - \rho(t) \tau(t).
\]

(3.3)

It is observed that the TREC content per naive cell will increase with increasing thymic export \(\theta(t)\) and decrease with increasing peripheral division \(\rho(t)\). Douek et al. [1] calculated TREC frequencies in naive CD4\(^+\) T cells in individu-
als aged 0 to 80 using total measured CD4$^+$ TREC and the percentage of naive T cells, assuming that memory (CD4$^+$ CD45R0$^+$) T cells contain only 2% of TREC. Their observations suggest that there is no significant change from the age of 0 to 20 (p=0.1) and, as in chapter 2, the mean TREC content per naive cell over this period, $\tau(t)$, is estimated to be $0.08 \pm 0.01$ (std. error). This conservation of TREC during the first two decades of life has also been observed in CD8$^+$ naive T cells [130] and in PBMCs [131].

The following expression for thymic export in terms of the average TREC content per naive cell and the rate of peripheral cell division is obtained from equation (3.3):

$$\theta(t) = \left( \tau(t) \rho(t) N(t) + \frac{d\tau(t)}{dt} N(t) \right) \frac{1}{c - \tau(t)}. \quad (3.4)$$

In order to extract a quantitative estimate for thymic export, $\theta(t)$, an estimate for both the number of cells added to the naive pool through peripheral cell division each day ($\rho(t) N(t)$) and the rate of change of the TREC content per naive cell with time ($d\tau/dt$) is required. It can be argued that the latter term is negligible since the average content per naive cell is approximately constant between ages 0-20 (see appendix 3.5.2). In the following section, Ki67 expression data is used to estimate the contribution of peripheral division.

**Using Ki67 expression to estimate the contribution of peripheral division**

Ki67 is a proliferation marker expressed from late stage G1 through to the end of mitosis [144]. Upon exit from cell cycle, it is rapidly degraded with a half-life of approximately 1 hour, independent of cell cycle position on exit from cycle [145]. Ki67 is therefore used to partition the naive CD4$^+$ population according to cell cycle status (at rest or cycling) and a two compartment model [119, 149] is used to estimate the rate of addition of new cells to the naive population through peripheral division. This model is illustrated in Figure 3.1.

Total naive T cell numbers are dependent on export of cells from the thymus, peripheral cell division and loss through death, differentiation or migration.
3.2 Mathematical model

out of the system. A simple dynamical model can be used to describe how total numbers of resting, $X(t)$, and dividing, $Y(t)$, naive CD4$^+$ T cells change with time:

\[
\frac{dX(t)}{dt} = \theta(t) + 2r(t)Y(t) - (a(t) + \delta_X(t))X(t), \tag{3.5}
\]

\[
\frac{dY(t)}{dt} = a(t)X(t) - (r(t) + \delta_Y(t))Y(t), \tag{3.6}
\]

where cells enter the resting naive cell compartment from the thymus at rate $\theta(t)$ (cells day$^{-1}$), resting cells enter cell cycle at rate $a(t)$, and are lost irreversibly from the naive resting pool at rate $\delta_X(t)$. The dividing population has its own rate of cell loss, $\delta_Y(t)$, and cells revert to the resting state at rate $r(t)$. All rates have units of days$^{-1}$. It is assumed that upon completion of cell cycle, two daughter cells will return to the naive resting pool.

In this model, the total number of naive cells is equal to the sum of the resting and dividing compartments and hence:

\[
\frac{dN(t)}{dt} = \frac{d[X(t) + Y(t)]}{dt} \tag{3.7}
\]

Combining equations (3.1), (3.5) and (3.6) gives:

\[
\theta(t) + \rho(t)N(t) - \delta(t)N(t) = \theta(t) + r(t)Y(t) - \delta_X(t)X(t) - \delta_Y(t)Y(t) \tag{3.8}
\]

where total loss of naive cells, $\delta(t)N(t)$, is assumed to be equal to the combined loss from the resting and dividing compartment, $\delta_X(t)X(t) + \delta_Y(t)Y(t)$. It is inferred from equation (3.8) that the number of cells added to the naive population through cell division in the periphery each day, $\rho(t)N(t)$, is equal to $r(t)Y(t)$. The rate of reversion of dividing cells to the resting pool, $r(t)$, can be interpreted as the inverse of average time spent expressing Ki67 during one
3.2 Mathematical model

complete cell cycle, $1/\Delta$ (an alternative modelling approach gives comparable results and is described in appendix 3.5.3):

Contribution of peripheral cell division (cells/day) = $\rho(t)N(t) = r(t)Y(t) \approx \frac{Y(t)}{\Delta}$

= \frac{\text{total number cells expressing Ki67}}{\text{duration of Ki67 expression}}.

Ki67 is expressed in late stage G1, S, G2 and M phase of cell cycle [144, 145]. The majority of variability in cell cycle duration is thought to arise from the length of G1, whereas the period of Ki67 expression coincides with the more deterministic 'B-phase' of cell cycle which is thought to be associated with a sequence of known physiological events of conserved length [146–148, 150]. Gett and Hodgkin [66] determined the average division time of stimulated naive CD4$^+$ T cells to be 12.4 hours ($\pm$1.0 hours), it can be argued that this reflects the minimum time taken for a naive cell to divide, and hence complete the deterministic phase of cell cycle, and let $\Delta$ be constant with mean 0.52 (day$^{-1}$). This is likely to be an upper bound on $\Delta$, since it also includes some time in interphase.

The total number of cells produced through cell division per day is estimated by combining data for Ki67 expression as a fraction of naive CD4$^+$ T cells, $y(t)$, with predicted total body naive cell numbers, $N(t)$:

Contribution of peripheral cell division (cells/day)

$$\rho(t)N(t) \approx \frac{y(t)N(t)}{\Delta} = \frac{y(t)\nu(t)V(t)}{0.02\Delta} \quad (3.9)$$

where $y(t)$ is the fraction of naive CD4$^+$ T cells expressing Ki67 such that $Y(t) = y(t)N(t)$ (Figure 3.2A, data taken from studies by Hazenberg et al. [2] and Douek et al. [1]); $\nu(t)$ is the naive CD4$^+$ T cell count per unit volume of blood [103] and $V(t)$ is the predicted total blood volume at age $t$. As in the previous chapter (Appendix 2.1), blood volume is estimated using a relationship between blood volume and body weight described by Linderkamp et al. [129], where standard body weight was estimated using standard CDC
growth data [128]. Lymphocytes in the blood are taken to represent 2% of all body lymphocytes [23], and Ki67 expression is assumed to be homogenous between blood and lymph nodes. The latter assumption is given some support by Fleury et al. [151] who found that the percentage of CD4$^+$ T cells expressing Ki67 in healthy individuals was 1.06% in the blood compared to 0.75% in the lymph nodes.

The 2.5 and 97.5 percentiles for the total daily contribution of peripheral division to the naive CD4+ T cell pool are obtained by combining the distributions of the three components. This procedure is outlined in appendix 3.5.4.

**An expression for thymic export**

An explicit expression for thymic export in terms of total naive cell numbers, naive cell TREC content and Ki67 expression is obtained by combining equations (3.4) and (3.9), as follows:

$$\text{Thymic Export (cells day}^{-1}) = \theta(t) \approx \frac{y(t) N(t) \tau}{\Delta (c - \tau)}, \quad (3.10)$$

where (as defined above) $y(t)$ is the fraction of naive CD4$^+$ T cells expressing Ki67, $N(t)$ is the total naive CD4$^+$ T cell population, $\Delta$ is the duration of Ki67 expression, and $\tau$ and $c$ are constants representing the average TREC content of the peripheral naive CD4$^+$ T cell population and thymocytes entering the peripheral naive population, respectively. Each of these parameters can be directly estimated using T cells sampled from peripheral blood.

### 3.3 Results

**Quantifying peripheral post-thymic production as a function of age**

Studies show that Ki67 expression in the naive CD4$^+$ T cell population declines from birth to age 30 years [1, 2]. Experimental Ki67 data from Douek et al. [1] and Hazenberg et al. [2] (Table 3.1) is combined and fitted to an exponential
decay function, \( a \exp(-ct) \) (age \( t \) in years), where \( a = 0.02 \) (0.012, 0.03) and \( c = 0.1 \) (0.03, 0.28) (95% confidence intervals in parentheses; Figure 3.2A).

The total number of dividing naive CD4\(^+\) T cells as a function of age, \( Y(t) \), was estimated by combining this decay function with an estimate of total peripheral naive CD4\(^+\) T cell numbers derived in chapter 2. This was used in equation (3.9) to calculate the expected total number of cells added to the naive population each day through peripheral cell division, between ages 0 to 20 years (Figure 3.2B). Although the proportion of naive CD4\(^+\) T cells expressing Ki67 declines monotonically with age, peak production in terms of absolute numbers of cells added to the naive pool each day occurs at age 1 year. The mean peripheral naive CD4\(^+\) T cell production is predicted to be \( 2.8 \times 10^9 \) (7.8 \times 10^8, 6.9 \times 10^9) cells per day at age 1, dropping to 6.8 \times 10^8 (2 \times 10^7, 4.1 \times 10^9) cells per day in individuals aged 20 (parentheses indicate estimated 2.5 and 97.5 percentiles at the population level). This corresponds to a continuous decline in the rate of division; 3.7% of the naive CD4\(^+\) T cell population is dividing per day at birth, this drops to 0.5% by age 20. The rate of decline in naive T cell production is comparable to the Ki67-independent estimates from the previous chapter; from 2% at birth to 0.2% at age 20. It is also in accord with estimates of naive production rates from deuterated glucose labelling studies in young adults of 0.2% [64].

Quantifying thymic export as a function of age

Ki67 expression and TREC content within the naive CD4\(^+\) T cell population is used to obtain a continuous estimate for thymic export from birth to age 20 years (Figure 3.3). The life history of a healthy thymus appears to comprise three distinct stages; the rate of thymic export increases during the first year of life, rapidly diminishes between the ages of 1 and 8 years, and then slowly declines from about age 8 years onwards. This trend directly follows the age-dependence of peripheral T cell production by proliferation (Figure 3.3B) and is a consequence of the growth of the naive compartment with age whilst maintaining constant TREC content within the naive pool [1].
Figure 3.2: A: Fraction of naive CD4\(^+\) T cells expressing Ki67 (data taken from Hazenberg et al. [2] & Douek et al. [1]) fitted to an exponential decay; \(0.02 \exp(-0.1t)\) (t measured in years), B: Estimated number of naive CD4\(^+\) T cells generated by peripheral division per day. Mean (solid line), 2.5% and 97.5% percentiles (dashed lines).

Using this approach the average thymus is predicted to export \(6.9 \times 10^8\) CD4\(^+\) T cells per day at birth (\(1.3 \times 10^8\), \(2.5 \times 10^9\)), a rate that doubles during the first 12 months of life to yield a daily export of approximately \(1.4 \times 10^9\) CD4\(^+\) T cells per day at 1 year (\(2.7 \times 10^8\), \(5 \times 10^9\)). This is consistent with histological studies showing that the human thymus continues to grow during the first year of life, reaching maximal volume at 1 year [8] and peak cellular density at 9 months [139, 152]; it is not altogether surprising as this period coincides with a time of rapid growth of the entire body and maturation of the immune system along with the decline in maternal antibody protection.

The biphasic decline in thymic export from ages 1 to 20 years is consistent with the decline predicted by the histological study of Steinmann et al. [8] (Figure 3.3B). There is a rapid contraction from age 1 to 8 years corresponding to an
average decline in output of 12% per year\(^1\) which is somewhat faster than the involution predicted by changes in the volume of TES with age. Following on from this rapid decay, there is a slower involution phase from about 8 years onwards which follows the involution of TES volume more closely. During this phase, thymic output drops from \(5.6 \times 10^8 (0.7 \times 10^9, 2.5 \times 10^9)\) to just under \(3.5 \times 10^8 (0.1 \times 10^8, 2.7 \times 10^9)\) CD4\(^+\) T cells per day by age of 20, corresponding to an average involution rate of 4% per year.

![Graph A: Estimated rate of thymic export of CD4\(^+\) T cells (cells/day): mean (solid line) and 2.5% and 97.5% percentiles (dashed lines).](image1)

Figure 3.3: A: Estimated rate of thymic export of CD4\(^+\) T cells (cells/day): mean (solid line) and 2.5% and 97.5% percentiles (dashed lines), B: Thymic involution predicted by mean thymic export (solid line) and relative volume of thymic epithelial space allowing for growth during first year of life, scaled to maximal volume (dashed line) [8].

### Thymic involution to age 60 years

The model is extended by considering the changes in naive CD4\(^+\) T cell numbers, TREC\(s\) and Ki67 expression in healthy subjects to age 60 years. A number of studies have reported that the size of the naive CD4\(^+\) T cell population and

\(^1\)average annual rate of involution, \(x\), estimated by \(\theta(t) = \theta(t_0)(1 - x)^{(t - t_0)}\)
fraction of naive CD4\(^+\) T cells expressing Ki67 remains fairly constant from age 20 to 60 years [1,70,102,103]. Meanwhile, the number of TREC per naive CD4\(^+\) T cell are observed to decline significantly from age 20 years onwards (Table 3.2; data from Douek et al. [1]). The model is used to interpret the data as before (equation 3.4), only naive TREC are allowed to vary with age, as shown in Figure 3.4C.

The model predicts that thymic output declines almost linearly from age 20 to 60 years; average output is projected to drop by \(\sim 96\%\) from just under \(3 \times 10^8\) at age 20 to \(\sim 1 \times 10^7\) cells per day by age 60 years; corresponding to an annual rate of involution of \(\sim 8\%\) per year. This is comparable to a \(\sim 95\%\) decline in the number of TREC per naive T cell over the same period [1]; an \(\sim 80\%\) decline in TES volume [8]; a \(\sim 90\%\) drop in the fraction of PTK7\(^+\) naive T cells [3]; a \(\sim 50\%\) decline in the fraction of CD31\(^+\) naive CD4\(^+\) T cells in the CD4\(^+\) T cells and a simultaneous \(\sim 2\)-fold drop in the number of TREC per CD31\(^+\) naive CD4\(^+\) T cell [56].

### 3.4 Discussion

Using mathematical modelling to analyse cell population dynamics, a quantitative estimate for thymic export is obtained using CD4\(^+\) naive T cell counts, TREC density and Ki67 expression within the naive population. It is shown that the number of cells exported by the thymus per day doubles during the first year of life and then declines in a biphasic manner throughout childhood; the most extensive decline in production is observed between the ages of 1 and 8. Following a slack period between the age of \(\sim 10\) and 20 years, thymic involution continues at a steady, almost linear rate, from age 20 to 60 years. In the previous chapter, the density of TREC within the naive population was modelled to infer a relationship between the relative contributions of thymic export and peripheral cell division to the naive CD4\(^+\) T cell population. The relative changes in naive production and loss with age were investigated based on the assumption that thymic export followed the involution of the TES. However, it was not clear that TES volume would necessarily directly correlate to the
Figure 3.4: A: Naive CD4$^+$ T cell density in blood (cells/µl), as described by Hue-necke et al. [103]; B: Fraction of Ki67$^+$ naive CD4$^+$ T cells, as measured by Hazen-berg et al. [2] and Douek et al. [1], fitted to a simple exponential decay; C: Observed TRECs per naive CD4$^+$ T cell, as measured by Douek et al. [1], fitted to a linear decay; D: Predicted thymic output (cell/day). Mean (solid line) and 2.5% and 97.5% percentiles (dashed lines).
number of cells exported to the periphery each day. In addition, assessing TES volume requires careful study of biopsied thymic material and is not a particularly tractable technique for assessing thymic export on an individual basis. In this work an alternative approach, relying on peripheral blood markers, is used to quantify thymic output.

Combined production of naive CD4⁺ T cells through cell division and thymic export is predicted to be \( \sim 1.0 \times 10^9 \) cells per day at age 20 years, this is comparable to estimates of total production in young healthy volunteers of \( 1.3 \times 10^8 \) naive CD4⁺ T cells per day as measured by *in-vivo* heavy water labelling studies [62], and \( 5.7 \times 10^8 \) cells per day obtained by deuterated glucose labelling [61]. The pattern of thymic involution predicted by the TES [8] is found to correlate well to our predicted change in thymic output to age 20 years (Figure 3.3B). From age 20 years onwards, the model predicts a rate of involution of thymic output of \( \sim 8\% \) per year that somewhat exceeds the decay predicted by TES volume of \( \sim 4\% \) per year, suggesting that the number of T cells exported may not be directly correlated to volume of thymic tissue.

The TREC concentration of thymic emigrants must be estimated in order to model TREC dynamics within the peripheral naive CD4⁺ T cell population. Previous studies suggest that this is stable with age from 0 to 60 years [52,132], although, the value of this concentration can not be directly measured and is a source of uncertainty in our estimates. For the purpose of this study, it is argued that this equals 0.25 based on the TREC content of recent thymic emigrants (CD31 expressing naive CD4⁺ T cells) in neonates [133] and the observation that 3 divisions occur within the thymus following the formation of sjTRECs [52]. An upper bound for the TREC concentration in thymic emigrants is determined from the TREC concentrations measured in CD4⁺ single positive thymocytes (0.6, ref [134]); this is a conservative estimate since proliferation is known to occur at the single positive stage of thymocyte development [153]. The choice of this parameter scales our estimates for thymic export, although the scaling is independent of age; taking the maximal value of this parameter to be 0.6 gives a three fold lower estimate for thymic export than our estimate.
Another potential source of uncertainty in our estimates as discussed above is the duration of Ki67 expression. This period coincides with the deterministic B-phase of cell cycle and it was assumed that it is fixed at approximately 12 hours [66], alternative experimental estimates for the length of CD4+ T cell cycle vary from 12 hours to 20 hours [67]. This parameter scales our estimates for both peripheral production and thymic export and is something that could be more accurately determined by experiment. This does not influence the predicted rate of decline in production with age; however, taking an upper and lower bound on Ki67 expression to be 20 and 12 hours, leads to predicted thymic export of $2.1 \times 10^8$ and $3.5 \times 10^8$ cells per day at age 20, respectively.

Residual expression of Ki67 on thymic emigrants is a possibility, however, the extent to which this will influence our estimates for peripheral production is limited by the rapid loss of Ki67 upon exit from cell cycle [145]; even if all cells exit the thymus immediately on completion of cell cycle, a half-life of 1 hour means that Ki67 expression resulting from thymic emigrants would still be negligible compared to expression resulting from peripheral division.

In this work, a method was derived for explicitly quantifying thymic export using parameters that can be directly measured from peripheral blood. When modelling in vivo lymphocyte dynamics there is always a need to translate the parameters that are observed in peripheral blood to the entire lymphatic system. In this study, an important assumption is made that the systems are homogeneous. This is justified by the observation that there is no significant difference between TREC concentrations in lymph nodes as compared to peripheral blood in healthy individuals [154], and Ki67 expression has also been observed to be similar in both compartments [155].

In order to estimate total body cell numbers it is assumed that at any given time only 2% of lymphocytes will be found in the blood. The decline in the frequency of naive T cells in blood with age is well-established [103,156] although there exists significant variation between individuals. This inter-individual
variation is compounded when estimates are scaled up for the lymphatic compartment and accounts for a large component of the variance that is predicted at the population level.

If our understanding of normal thymic export in health is limited, then the role of the thymus in disease is even less understood. Premature involution of thymic volume is associated with rapid disease progression in human immunodeficiency virus (HIV) infected children [157] but it is not known if this reduction in thymic volume reflects reduced thymic export and some older HIV-infected individuals show increases in thymic volume compared to age-matched healthy controls [158]. A better measure of the number of cells exported by the thymus is required to properly understand the role of the thymus in disease progression and T cell reconstitution following antiretroviral therapy. TREC levels are decreased in HIV-infected individuals [1] but it was shown that this largely reflects an increase in the rates of peripheral division rather than decreased thymic output [53, 159]. Here a method is presented to estimate thymic export using peripheral TREC and Ki67 expression data in healthy individuals that allows us to explore the natural involution of the thymus with age and provides a step towards better understanding the contribution of the thymus to the nature of T cell reconstitution occurring following chemotherapy, hematopoietic stem cell transplant, thymic implant or treatment with anti-retroviral therapy in HIV.

3.5 Appendices

3.5.1 Derive an expression for $\frac{d\tau(t)}{dt}$

Let $\tau(t)$ equal to $T(t)/N(t)$ and consider the derivative of $\tau(t)$:

$$\frac{d\tau(t)}{dt} = \frac{N(t)\frac{dT(t)}{dt} - T(t)\frac{dN(t)}{dt}}{N(t)^2}$$  \hspace{1cm} (3.11)
3.5 Appendices

Equation (3.3) is obtained using the derivative of $N(t)$ and $T(t)$ as determined by equations (3.1) and (3.2):

$$\frac{d\tau(t)}{dt} = \frac{N(t)(c\theta(t) - \delta(t)T(t)) - T(t)(\theta(t) + \rho(t)N(t) - \delta(t)N(t))}{N(t)^2}$$

(3.12)

$$\frac{\theta(t)}{N(t)}(c - \tau(t)) - \rho(t)\tau(t)$$

(3.13)

3.5.2 Validate assumption that $\frac{d\tau(t)}{dt} = 0$

From equations (3.4) and (3.2) (an expression for thymic export is obtained in terms of the size of the total naive pool, $N(t)$, the proportion of dividing cells, $y(t)$, and the rate at which the average TREC content per naive cell changes with time:

$$\theta(t) = \left(\frac{y(t)\tau(t)}{\Delta} + \frac{d\tau(t)}{dt}\right) \frac{N(t)}{c - \tau(t)}$$

(3.14)

where $c$ is the average TREC content of thymocytes leaving the thymus and $\Delta$ is average length of Ki67 expression, both of which are assumed to be constant. The TREC content per naive CD4$^+$ T cell was found to be approximately fixed from birth to age 20 years by Douek and colleagues [1]. A number of studies suggest that the average per cell TREC content is conserved during the first two decades of life [130,131]. So we let $\tau(t)$, the number of TRECS per naive CD4$^+$ T cell, be constant with mean $0.08 \pm 0.01$ (s.e.m.).

Although it is assumed that there exists no significant change in $\tau$ with age, there is considerable variation between individuals so $\tau$ is fitted to a linear decay and the 95% confidence interval of the slope, or equivalently $\dot{\tau}(t)$, is found to be (-1.7x10$^{-5}$, 2x10$^{-6}$).

The assumption that $\dot{\tau}(t) \approx 0$ is justified by considering that minimal Ki67 expression within the naive CD4$^+$ T cell population occurs at age 20 and is of the order 0.2%, this is combined with estimates for $\Delta$ ($\approx 0.5$, ref [66]), and $\tau$ ($\approx 0.08$, ref [1]) to numerically approximate $\text{Min}[y(t) \tau/\Delta] \approx 3x10^{-4}$. $\text{Max}[\dot{\tau}(t)]$ is esti-
mated from the 95% confidence interval as described above to be of the order 1.7x10^{-5}, so \( \dot{\tau}(t) \) is approximately 20-fold smaller than the first term, \( y(t)\tau/\Delta \), in equation (3.14). This difference is even more pronounced in younger children where up to 2% of naive cells are dividing at any given time, and \( \dot{\tau}(t) \) is greater than 100-fold smaller than the first term, hence the \( \dot{\tau}(t) \) term is ignored and equation (3.14) becomes

\[
\theta(t) = \frac{y(t) \, N(t) \, \tau}{\Delta \, (c - \tau)}.
\]

(3.15)

In Figure 3.5 thymic export is estimated assuming that TREC frequency in the naive CD4^+ T cell pool declines continuously, from approximately 0.1 at birth to 0.001 at age 80, and is found to be comparable to our original results where it was assumed that the naive TREC frequency was constant to age 20 and equal to 0.08. The 100 fold decrease in naive TREC frequency over 80 years corresponds to a rate of change in \( \tau \) per day of 3 \times 10^{-6} using the same argument as above this is 100 fold smaller than the first term in equation (3.14) and the difference between the two estimates is largely due to the different values for frequency of TRECs per naive cell, \( \tau \).

![Figure 3.5: Thymic export calculated under the assumption that frequency of TRECs per naive CD4^+ T cell decline continuously from approximately 0.1 at birth to 0.001 at age 80 (dashed line), compared to thymic export calculated assuming the value of TRECs per naive cell is 0.08 and remains constant until age 20 (solid line).]
3.5.3 Alternative modelling approach to estimate contribution of peripheral cell division

Smith and Martin [146] proposed a model of cell cycle divided into two phases; a stochastic A-phase of variable length, representing the G1 stage of cell cycle, followed by a more deterministic B-phase including the S, G2 and M stages of cycle. Ki67 is a cell cycle marker that is expressed from late stage G1 and is rapidly degraded post mitosis [144, 145], and hence provides a fairly good measure of the number of cells in B-phase. This model is adapted to interpret Ki67 expression within the naive CD4$^+$ T cell population.

It is estimated that cells take $\Delta_B$ days to complete B-phase, where time taken is assumed to be relatively conserved, irrespective of cell type or age. Gett and Hodgkin [66] determined the average division time for a naive CD4$^+$ T cell to be 12.4 (±1.0 hours).

It is assumed that resting cells (Ki67$^-$) in an individual of age $t$ transition into B-phase at a rate $a(t)$. This is modelled as an exponential processes, and so $1/a(t)$ is approximately the expected time to entry to B-phase for a given resting (Ki67-) naive CD4$^+$ T cell. Define $S(t)$ to be the number of naive cells produced through cell division in the periphery each day. Cells produced between time $t$ and $t - 1$ must enter the B phase of cycle between $t - 1 - \Delta_B$ and $t - \Delta_B$, where time is measured in units of days:

$$S(t) = \int_{t-\Delta_B-1}^{t-\Delta_B} N(i)a(i)e^{-\gamma\Delta_B} di,$$

where a cell takes $\Delta_B$ days to complete B-phase, $N(i)$ is the size of the total naive cell population, and $a(i)$ is defined previously. At any point in time, $i$, it is assumed that $N(i)a(i)$ cells will enter the deterministic phase of cell cycle and the probability of successfully survive B-phase and completing mitosis is estimated to be $\exp(-\gamma\Delta_B)$, where $\gamma$ is the death rate of naive cells in cycle. It is assumed that $N(i)$ and $a(i)$ do not change significantly over the period of a few days. Hence the function to be integrated becomes approximately a constant.
and the number of naive cells produced through division in the periphery each day can be expressed as

\[ S(t) = N(t)a(t)e^{-\gamma \Delta_B} \]  

(3.17)

where \( N(t) \) and \( a(t) \), represent \( N(i) \) and \( a(i) \) for \( i \in (t - \Delta_B - 1, t - \Delta_B) \), respectively.

Using the same approach a related expression can be derived for the number of cells that express Ki67 at any given time, \( Y(t) \):

\[ Y(t) = \int_{t-\Delta}^{t} N(i)a(i)e^{-\gamma(t-i)} \, di \]  

(3.18)

where \( \Delta \) is the duration of Ki67 expression. As before it is assumed \( N(t) \) and \( a(t) \) are constant over the period of a few days, and hence the number of naive cells expressing Ki67 at any given time, \( t \), can be described as follows:

\[ Y(t) = \frac{N(t)a(t)}{\gamma} \left( 1 - e^{-\gamma \Delta} \right) \]  

(3.19)

In order to find an expression for total cell production per day in terms of Ki67 expression, the duration of Ki67 expression, \( \Delta \), is estimated to be approximately equal to the duration of B-phase, \( \Delta_B \), to give the following expression for cell production per day from equations (3.17) and (3.19):

\[ S(t) = \frac{Y(t)\gamma}{e^{\gamma \Delta} - 1} \approx \frac{Y(t)}{\Delta + \frac{\gamma^2 \Delta^2}{2} + O \left[ \frac{\gamma^2 \Delta^3}{3!} \right]} \]  

(3.20)

where \( \gamma \) is the rate of loss of naive cells from cycle. There is currently no estimate for this term from the experimental literature that we are aware of, however it is known to lie between 0 and 1. The last terms in the above expression are ignored since they are at least fifty fold smaller than \( \Delta \); \( \Delta \approx 0.5 \) (days) and \( \gamma < 1 \) implies that \( \gamma^2 \Delta^3/3! < \Delta/48 \).
Applying this definition of peripheral production to equation (3.4) gives:

\[ \theta(t) = \frac{y(t) N(t) \tau}{(c - \gamma^2/(\Delta + \gamma^2/2)) (c - \tau)} \approx \frac{y(t) N(t) \tau}{(\Delta + \gamma^2/2) (c - \tau)} \] (3.21)

where dividing cells represent such a small proportion of the overall naive pool that the rate of loss from this subpopulation, \( \gamma \), may well be significantly higher than the average rate of loss from the bulk naive CD4\(^+\) T cell population. In Figure 3.5 thymic export is calculated for the extreme values of \( \gamma \) and it is found that the predicted output is fairly robust to the value of \( \gamma \). Using this approach to model Ki67 expression gives comparable estimates for thymic export to the approach described previously in the methods (compare Figures 3.3 and 3.6).

![Graph showing thymic export estimated using an alternative modelling approach](image)

**Figure 3.6:** Mean thymic export estimated using an alternative modelling approach where \( \gamma = 0 \) (solid line), 0.5 (dashed line), or 1 (dotted line) in equation (3.21).

### 3.5.4 Modelling distribution of thymic export function

The distribution of total body naive cells, \( N(t) \), and TREC content per naive cell, \( \tau(t) \), is modelled elsewhere in appendix 2.5.1.

A simple exponential decay model, \( y(t) = a \exp(-ct) \) (\( t \) measured in years) is used to describe the experimentally observed frequency of naive CD4 T cells in blood that are Ki67\(^+\) as a function of age. \( R \) is used to determine the least-square estimates of the model parameters. A distribution is constructed for \( y(t) \) by considering the variation in the predicted parameters; \( a \) is assumed to
be normally distributed with mean 0.019 and variance 0.005, \( c \) has a mean of 0.099 (95% confidence interval: (0.026, 0.271)) and is best fit to a lognormal distribution with parameters \( \mu = -2.45 \) and \( \sigma = 0.56 \).

Average naive cell cycle duration, \( \Delta \), is assumed to be normally distributed with mean 0.52 days and variance 0.05 [66].

Thymic export \( \theta(t) \) is a non-linear function of \( N(t) \), \( y(t) \), \( \tau \), \( \Delta \) and \( \dot{\tau}(t) \), and so a Monte Carlo approach is used to construct an approximate, empirical distribution for \( \theta(t) \).
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Table 3.1: Observed percentage of naive CD4$^+$ T cells expressing Ki67 in healthy individuals aged 0 to 70 years. Data from Hazenberg et al. [2] & Douek et al. [1])
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Table 3.2: Observed frequency of TREC per naive CD4$^+$ T cell in healthy individuals aged 0 to 60 years. Data from Douek et al. [1]
Chapter 4

Inter-cellular variation in the residency time of PTK7$^+$ naive CD4$^+$ T cells
Abstract

Protein tyrosine kinase 7 (PTK7) has been identified as a surrogate marker of recent thymic emigration. The aim of this chapter is to explain the long-term persistence of PTK7$^+$ naive CD4$^+$ T cells in thymectomised individuals and explore the residency time of thymic emigrants within the PTK7$^+$ state. It is shown that incomplete thymectomy alone can not fully explain the residual population of PTK7$^+$ T cells and that the average rate of PTK7$^+$ T cell loss must decline following thymectomy. Inter-cellular variation in the time taken to differentiate from PTK7$^+$ to PTK7$^-$ T cells can explain the bi-phasic decline in PTK7$^+$ T cells following thymectomy. The mathematical model described here predicts that a heavy-tailed distribution of PTK7 residency times, with an expected duration of PTK7 expression of $\sim$23 days, would be required to explain the experimental observations. The implications of this model are: (a) thymectomy preferentially depletes ‘shorter-lived’ cells leading to an accelerated increase in the average post-thymic age of remaining PTK7$^+$ T cells following thymectomy; and (b) more-persistent ‘veteran’ cells will preferentially accumulate over a number of decades leading to an increase in the average post-thymic age of PTK7$^+$ T cells in older individuals. Suggesting that, despite the immature phenotype, PTK7$^+$ T cells do not necessarily represent a population of recent thymic emigrants, particularly in thymectomised and elderly subjects. These finding suggest that the impact of thymectomy may go beyond the observed decline in cell numbers.
4.1 Introduction

The thymus plays an important role in maintaining T cell numbers and a diverse T cell receptor repertoire throughout the lifetime of an individual. In humans, recent thymic emigrants (RTEs) are commonly identified by cell surface markers expressed on circulating T lymphocytes. CD31 (platelet endothelial cell adhesion molecule-1, PECAM-1) and PTK7 (protein tyrosine kinase 7) are two surrogate markers commonly used to identify naive CD4+ T cells in humans that have recently emigrated from the thymus [3,55]. PTK7 is most highly expressed on least mature, double-negative (CD4−CD8−), thymocytes and is progressively lost as cells transition to the more mature single-positive (CD4+CD8− or CD4−CD8+) stage of thymic development [3]. It is thought that circulating PTK7+ CD31+ naive CD4+ T cells are immediate peripheral descendants of single-positive thymocytes and are precursors of more established PTK7−CD31+ naive CD4+ T cells. Thymic emigrants appear to first lose expression of PTK7 and then CD31 in an antigen-independent manner as part of the standard passage of T cells joining the peripheral population (Figure 4.1). This is supported by the following observations: (a) the PTK7+ CD31+ subset is contained within the peripheral CD31+ naive CD4+ population; and (b) the number of signal joint TCR gene excision circles (sjTRECs) (stable non-replicative DNA products of intrathymic V(D)J recombination) [52] is approximately 2.5-fold higher in PTK7+ than PTK7− naive CD4+ T cells but less frequent than in single positive thymocytes.

The natural progression of naive CD4+ T cells entering the peripheral population, from PTK7+ CD31+, through PTK7− CD31+, to PTK7− CD31− naive T cells, is poorly understood. In vitro, cytokine-induced division results in the progressive loss of surface PTK7 expression [3] while preserving CD31 expression [141,142]; however, the signals that induce a shift in phenotype in vivo are unknown and in particular there are few studies exploring the role of PTK7 within T cells. PTK7 has been more extensively studied for its role in gastrulation and neurulation [160]; it is a co-receptor in the non-canonical WNT pathway and controls the transcription of genes important to tissue polarity,
cell adhesion and motility [161, 162].

Figure 4.1: Post-thymic maturation of T cells entering the circulating naive CD4$^+$ T cell population.

In this chapter the residency time of thymic emigrants within the peripheral PTK7$^+$ naive CD4$^+$ T cell population is considered as this will determine how well the marker can be applied as a correlate of thymic activity. It is not possible to directly measure, *in vivo*, the time that a T cell leaving the thymus will spend in the PTK7$^+$ naive CD4$^+$ T cell state, instead our knowledge of this process relies upon indirect observations of, for example, the fraction of circulating PTK7$^+$ naive CD4$^+$ T cells that remain when *de novo* production ceases, i.e. following thymectomy. Haines and colleagues observed that following an initial drop, thymectomised subjects were able to maintain a stable residual population of PTK7$^+$ naive CD4$^+$ T cells (so-called RTEs) for at least 6 months post-thymectomy [3]. The aim of this study is to explain the long-term persistence of PTK7$^+$ naive CD4$^+$ T cells in thymectomised individuals with a view to better defining the early PTK7$^+$ stage of T cell development and to ultimately better understand how thymic emigrants are incorporated into the peripheral naive T cell pool.
Theoretically, the persistence of PTK7$^+$ naive CD4$^+$ T cells might reflect: (a) residual thymic production; (b) increased peripheral expansion of PTK7$^+$ T cells; and / or (c) increased persistence of peripheral PTK7$^+$ T cells. Here a mathematical approach is used to show that incomplete thymectomy alone is unable to explain the residual PTK7$^+$ naive CD4$^+$ T cell population. It is not clear how compensatory mechanisms might operate to maintain PTK7$^+$ naive CD4$^+$ T cells in the absence of thymic production. Increased peripheral division might explain the experimental observations, however, in vitro at least, cytokine-induced division has been shown to result in the loss of PTK7 expression [3] suggesting that the extent to which division is capable of maintaining immature PTK7$^+$ naive CD4$^+$ T cells might be limited. Here it is shown that a heavy-tailed distribution of PTK7$^+$ T cell residency times, i.e. one where the survivorship of cells within the PTK7$^+$ state decays slower than exponentially, can explain the persistent PTK7$^+$ T cell population. A mathematical model is used to explore the consequences of this inter-cellular variation in residency times for the PTK7$^+$ T cell population in thymectomised and aged individuals. The findings suggest that, despite the immature phenotype, PTK7$^+$ T cells do not necessarily reflect recently emigrated T cells, particularly in thymectomised and elderly subjects.

4.2 Mathematical model

A survival model is used to explore the residency time, or ‘life expectancy’, of thymic emigrants within the PTK7$^+$ naive CD4$^+$ T cell population and to investigate the homeostatic processes that sustain the T cell pool following thymectomy. To study how long a cohort of thymic emigrants will persist in the circulating PTK7$^+$ naive CD4$^+$ T cell population, we let the cumulative distribution of residency times, $F(t)$, represent the fraction of cells from a given cohort of T cells leaving the thymus, in an individual aged $t$, that will remain in the circulating PTK7$^+$ naive CD4$^+$ T cell population $x$ days post-export. $F(t)$ can be described as a survivorship function and represents the fraction of PTK7$^+$ naive CD4$^+$ T cells that are not lost to differentiation, death, or migration out
4.2 Mathematical model

of the peripheral PTK7\(^+\) compartment. Studies have shown that the loss of recently produced naive CD4\(^+\) T cells through cell death or migration out of the peripheral system is negligible over labelling periods of up to 200 days \[62,64\], suggesting that the loss of PTK7\(^+\) naive CD4\(^+\) T cells, at least during the first 6 months after thymic export, will be almost exclusively associated with post-thymic maturation into the PTK7\(^-\) naive T cell population.

Under normal physiological conditions, the frequency of PTK7\(^+\) naive CD4\(^+\) T cells in an individual aged \(t\), \(X(t)\), is determined by a convolution of thymic output and the survivorship of those emigrants within the circulating PTK7\(^+\) naive CD4\(^+\) T cell population, as follows:

\[
X(t) = \int_0^t \theta(a) F_a(t) da. \tag{4.1}
\]

Where, \(\theta(a)\) is the rate at which PTK7\(^+\) naive CD4\(^+\) T cells are exported by the thymus (cells/day), in an individual aged \(a\); \(F_a(x)\) is the fraction of thymic emigrants that is predicted to persist in the peripheral PTK7\(^+\) naive CD4\(^+\) T cell population \(x\) days, in an individual aged \(a\). (All units of time and age are measured in days.) In the interests of simplicity it is assumed that division within the PTK7\(^+\) subpopulation is negligible. This assumption is justified by the observed loss of PTK7 expression on naive cells following division \textit{in vitro} \[3\].

In appendix 4.5.1 the model is extended to include a constant rate of division within the PTK7\(^+\) T cell pool and it is shown that the results that follow are robust to this background division.

### Modelling thymectomy

The general survival model is adapted to describe the natural decline of PTK7\(^+\) naive CD4\(^+\) T cells following thymectomy, in the absence of compensatory mechanisms such as increased proliferation or decreased cell loss. The size of the PTK7\(^+\) naive CD4\(^+\) T cell population, \(X^*(t)\), following thymectomy at age \(t_0\), can be described by allowing normal thymic production to age \(t_0\) but considering the fraction of cells that persist to some time of observation \((t > t_0)\)
4.2 Mathematical model

post-thymectomy, as follows:

\[ X^*(t) = \int_{0}^{t_0} \theta(a)F_a(t-a)da + \int_{t_0}^{t} p\theta(a)F_a(t-a)da \]  \hspace{1cm} (4.2)

where \( p \) represents the fraction of residual thymic production that continues post-thymectomy (\( 0 \leq p < 1 \)). At this stage no assumptions are made about the functional form of the survivorship function \( (F_a(t)) \). A simple exponentially decay would imply a constant rate of transition from PTK7\(^+\) to PTK7\(^-\) naive CD4\(^+\) T cells, while divergence from the simple exponential survivorship function would suggest that there exists some variation in the rate of transition.

Variation model

The variation model describes a scenario where the persistence of PTK7\(^+\) naive CD4\(^+\) T cells following thymectomy is a natural consequence of the normal variation in the rate at which cells transition from PTK7\(^+\) to PTK7\(^-\). A mathematical model can be used to estimate the underlying survivorship function required to describe the clinical observations under the variation model as follows.

Equation (4.2) can be rewritten to compare the number of PTK7\(^+\) naive CD4\(^+\) T cells in a healthy individual aged \( t \), \( X(t) \), to the expected number of PTK7\(^+\) naive CD4\(^+\) T cells in an individual that undergoes thymectomy at age \( t_0 \), \( X^*(t) \), where \( t \geq t_0 \), as follows:

\[ X^*(t) = X(t) - \int_{t_0}^{t} (1 - p)\theta(a)F_a(t-a)da. \]  \hspace{1cm} (4.3)

According to the variation model, the frequency of PTK7\(^+\) T cells post-thymectomy is simply equivalent to the size of the population in a non-
4.2 Mathematical model

thymectomised, aged-matched individual less the cells that would have been
produced from time \( t_0 \) to a later observation time, \( t \). In order to extract
the survivorship function, \( F_a(x) \), that is required to explain observed PTK7+
T cells post-thymectomy, consider the rate of change of cell numbers post-
thymectomy:

\[
\frac{dX^*(t)}{dt} = \frac{dX(t)}{dt} - \lim_{\Delta t \to 0} \frac{\int_{t_0}^{t_0 + \Delta t} (1 - p)\theta(a)F_a(t + \Delta t - a)da - \int_{t_0}^{t} (1 - p)\theta(a)F_a(t - a)da}{\Delta t}
\]  

(4.4)

where \( dX(t)/dt \approx 0 \), since the observed change in PTK7+ naive CD4+ T cell
numbers is negligible in non-thymectomised children over a 6 month period
[3], and a change of variables, \( a = a' + \Delta t \), can be used to rewrite the first
integral, as follows:

\[
\frac{dX^*(t)}{dt} \approx -(1 - p) \lim_{\Delta t \to 0} \frac{\int_{t_0}^{t} \theta(a' + \Delta t) F_{a' + \Delta t}(t - a')da' - \int_{t_0}^{t} \theta(a) F_a(t - a)da}{\Delta t}
\]  

(4.5)

Equation (4.5) can be simplified by assuming: (a) \( \theta(a' + \Delta t) \approx \theta(a') \), it is
reasoned that thymic production diminishes over a number of decades and
hence the change over some small period \( \Delta t \) is likely to be negligible; and (b)
\( F_{a' + \Delta t}(t - a') \approx F_{a'}(t - a') \), where the survivorship function of PTK7+ T cells is
not likely to be significantly different for cohorts of thymic emigrants leaving
the thymus \( \Delta t \) days apart. The equation becomes:

\[
\frac{dX^*(t)}{dt} \approx -(1 - p) \lim_{\Delta t \to 0} \frac{\int_{t_0}^{t} \theta(a) F_a(t - a)da}{\Delta t}
\]  

\[
\approx -(1 - p)\theta(t_0)F_{t_0}(t - t_0)
\]  

(4.6)

Since all T cells leaving the thymus are assumed to enter the PTK7+ population
at day 0, the boundary condition is \( F_{t_0}(0) = 1 \) and the equation becomes:
\[ \theta(t_0) \approx -\frac{1}{1 - p} \frac{dX^*(t_0)}{dt}. \]  
(4.7)

Hence, we obtain the following expression for survivorship function:

\[ F_{t_0}(i) \approx \frac{1}{dX^*(t_0)} \frac{dX^* (i \text{ days post thymectomy})}{dt}. \]  
(4.8)

Using this expression, we can infer the underlying distribution of residency times of peripheral PTK7\(^+\) naive CD4\(^+\) T cells leaving the thymus at age \( t_0 \) using the observed rate of change in PTK7\(^+\) naive CD4\(^+\) T cell numbers post-thymectomy.

### 4.3 Results

Residual thymic output is not sufficient to explain the residual PTK7\(^+\) naive CD4\(^+\) T cell population post-thymectomy

First, it is investigated whether residual thymic output alone is able to explain the long term presence of PTK7\(^+\) naive CD4\(^+\) T cells following thymectomy, in the absence of inter-cellular variation in PTK7 residency times or compensatory mechanisms such as a density-dependent rates of cell loss and / or proliferation. In order to do this, the most general survival model is used to consider the post-thymectomy dynamics in the absence compensatory mechanisms (equation (4.2)). The influence of inter-cellular variation is removed by assuming a constant rate of transition from PTK7\(^+\) to PTK7\(^-\) T cells, of \( \lambda > 0 \), such that the survivorship function becomes a simple exponential decay \( (F_a(t) = \exp[-\lambda t]) \).

Equation (4.2) describes the frequency of PTK7\(^+\) naive CD4\(^+\) T cells following thymectomy and can be re-written as follows:
\[ X^*(t) = (1 - p) \int_0^t \theta(a) F_a(t - a) da + p \int_0^t \theta(a) F_a(t - a) da \]

\[= (1 - p) \int_0^t \theta(a) \exp[-\lambda(t - a)] da + p \int_0^t \theta(a) \exp[-\lambda(t - a)] da \]

\[= A \exp[-\lambda t] + pX(t) \]

\[(4.9) \rightarrow pX(t) \text{ as } t \rightarrow \infty.\]

where \( A = (1 - p) \int_0^t \theta(a) \exp[\lambda a] da \), is constant with respect to \( t \). It can be seen that under this simple model, PTK7+ T cell numbers will exponentially drop to \( pX(t) \), where \( p \) represents the fraction of thymic production that continues after thymectomy and \( X(t) \) represents the expected number of PTK7+ T cells in a normal, non-thymectomised individual of the same age. In Figure 4.2, the incomplete thymectomy model alone is used to predict the change in PTK7+ naïve CD4+ T cells post-thymectomy for varying fractions of residual thymic production, \( p \). In the absence of inter-cellular variation in residency times or compensatory mechanisms in response to thymectomy, residual thymic output of the order of \( \sim 15\% \) and \( \sim 45\% \) is required to explain experimental observations made by Haines et al. [3] in children thymectomised at age 2 and 14 years, respectively (Figure 4.2 B & C).

Although complete thymectomy is unlikely to be 100% efficient, the analysis presented here suggests that residual thymic production alone would not be sufficient to explain the observed residual PTK7+ population post-thymectomy. Haines and colleagues [3] followed the progress of subjects with myasthenia gravis where thymectomy was described as clinically complete. Typically, short-term completion studies in patients who have undergone complete thymectomy for treatment of myasthenia gravis reveal <5% residual thymic tissue [163]. Alternative sources of thymic production could include ectopic thymi. For example, Terszowski and colleagues [164] presented evidence for a functional second thymus in the mouse neck. Although the structure of both, chest and neck, thymus were found to be similar, a thymic lobe in the neck
4.3 Results

Figure 4.2: Simulating PTK7+ naive CD4+ T cells post-thymectomy for varying fractions of residual thymic production, \( p_t \), in the absence of compensatory division or varying rates of cell loss due either to density-dependent survival or heterogeneity in the residency times. A: Array of feasible exponential PTK7 survivorship functions (corresponding to a constant exponential rate of transition from PTK7+ to PTK7− naive T cells). B & C: Simulated post-thymectomy dynamics in a typical 2 and 14 year old, reconstructed using equation (4.9) where the fraction of residual thymic production is set to be 0, 0.1, 0.2 and 0.45, and each curve corresponds to a survivorship function identified in 4.2A. Estimates for median thymic export are as determined in chapter 3. Actual observations by Haines & colleagues [3] (filled circles).
was found to carry $\approx 1.6 \times 10^5$ thymocytes compared to $\approx 1 \times 10^8$ in the thoracic thymus. Additionally, the thoracic thymus was observed to be multi-lobulated, whereas the neck thymus appeared single lobed and at most 3 ectopic thymic organs were observed. If it is assumed that thymic output is comparable to the number of thymocytes, this suggests that the relative contribution of secondary thymic tissue is at least 100-fold smaller than the primary thoracic thymus. In combination, these observations suggest that residual thymic production might realistically be of the order of less than 5% which is somewhat less than the $\sim 15$-$45\%$ required to explain the experimental observations.

The average per-cell rate of loss of PTK$7^+$ naive CD$4^+$ T cells declines with increasing time post-thymectomy

Since incomplete thymectomy alone is unable to explain the long term persistence of PTK$7^+$ naive CD$4^+$ T cells following thymectomy, the role of extended PTK$7^+$ naive CD$4^+$ T cell persistence is considered. A simple ordinary differential equation model is used to show that the average rate at which cells transition from PTK$7^+$ to PTK$7^-$ naive CD$4^+$ T cells must decline after thymectomy. If thymectomy is assumed to be complete, then the following expression determines the size of the PTK$7^+$ naive CD$4^+$ T cell population, $X^*(t)$, after thymectomy:

$$\frac{dX^*(t)}{dt} = -\delta(t)X^*(t)$$  \hspace{1cm} (4.10)

where $\delta(t)$ is the average per-cell rate of loss of cells from the PTK$7^+$ naive CD$4^+$ T cell population, at time $t$ after thymectomy. Clinical observations suggest that the PTK$7^+$ T cell population, $X^*(t)$, approaches a new (non zero) set point following thymectomy [3], hence $\delta(t)$ must approach zero with after thymectomy to explain the decline in net change in PTK$7^+$ T cells per day ($\frac{dX^*(t)}{dt}$) to zero [3].

There are at least two distinct ways in which a decline in the average rate of loss can be explained. One possibility is a model in which the rate of transi-
tion, from PTK7$^+$ to PTK7$^-$ naive T cells, declines homogeneously across the entire PTK7$^+$ pool with time, perhaps as a function of population size. Density-dependent growth and survival are commonly applied in ecological models to explain the maintenance of population numbers and are typically thought to arise from some form of competition for limited resources or predation. In this situation loss of PTK7$^+$ naive CD4$^+$ T cells is thought to predominantly involve transition from the less mature PTK7$^+$ to more established PTK7$^-$ T cell pool and the mechanisms that might lead to a density-dependent length of stay in immature PTK7$^+$ stage of the T cell lifecycle are not at all clear. A density-dependent rate of transition would suggest that PTK7$^+$ T cells require some stimuli to remain in the PTK7$^+$ state, ie. differentiation from PTK7$^+$ to PTK7$^-$ is driven by the loss of some signal, perhaps related to IL-7 availability. At lower cell densities there would be plenty of the required stimuli resulting in a lower rate of PTK7$^+$ T cell loss, while more cells would result in more competition for the stimuli and an increase in the rate of PTK7$^+$ T cell loss. If the opposite was true, ie. differentiation from PTK7$^+$ to PTK7$^-$ was positively driven by some maturation signal, then at lower cell densities there would be potentially fewer PTK7$^+$ T cells competing for such stimuli leading to an increase in the rate of transition, which is the opposite of the required relationship between PTK7$^+$ T cell density and loss.

An alternative to this homogeneity model, is a variation model where there exists natural inter-cellular variation in PTK7$^+$ naive CD4$^+$ T cell residency times. Here, the initial decline in PTK7$^+$ T cells following thymectomy would reflect the loss of intrinsically “shorter-lived” cells that rapidly transition into the PTK7$^-$ T cell compartment immediately after export, while the second relatively stable phase of thymectomy reflects the behaviour of the naturally “more-persistent” cells.

In the work that follows, the distribution of residency times that is required to fit the variation model is estimated and the consequences of such a distribution for the natural aging process are examined. Although both models can explain the clinical changes in PTK7$^+$ naive T cell numbers, it is shown that
inter-cellular variation has some serious implications for the post-thymic age of residual PTK7\(^+\) T cells in thymectomised individuals.

**Expected residency time of PTK7\(^+\) naive CD4\(^+\) T cells \(~\sim\~23.3\) days.**

As the number of observations following thymectomy are fairly limited [3], measurement error of ± 20 cells/\(\mu\)l is allowed for and an array of standard bi-exponential curves is used to define the feasible observation space (Figure 4.3A & B). A method for estimating the distribution of residency times (or equivalently survivorship function) that is required to describe a given decline in PTK7\(^+\) naive CD4\(^+\) T cells under the variation model is derived in equation (4.8). In Figure 4.3C, the survivorship functions that correspond to the bi-exponential curve within the feasible space are explored.

It is assumed that a per-cell rate of loss of 1(/day) gives a reasonable upper bound on the rate of transition from PTK7\(^+\) to PTK7\(^-\) naive T cells. This limit determines an upper bound on the decay of PTK7\(^+\) naive CD4\(^+\) T cells post-thymectomy and a minimal survivorship function for thymic emigrants within the PTK7 compartment (red lines in Figure 4.3A, B & C).

The maximal survivorship function is identified by the feasible decay of PTK7\(^+\) T cells post-thymectomy that corresponds to the maximal survivorship to day 250 (Max\([F_a(250\text{ days})]\)), since experimental observations only extend to 250 days post-thymectomy (blue line in Figure 4.3A & B). The maximal survivorship function corresponds to the persistence of approximately \(~\sim\~0.4\)% of thymic emigrants within the PTK7\(^+\) compartment 250 days after export in 2 year old, and \(~\sim\~12\)% at age 14 years (Figure 4.3C).

The model assumes that the survival of PTK7\(^+\) naive CD4\(^+\) T cells post-thymectomy reflects the normal physiological behaviour of cells in the absence of compensatory mechanisms. In the presence of additional production or extended PTK7 lifespans in response to thymectomy, the model will overstate the ‘normal’ persistence of PTK7\(^+\) naive CD4\(^+\) T cells and hence this upper bound
is a conservative limit on PTK7 persistence and is robust to different models of post-thymectomy dynamics.

The feasible survivorship functions identified in Figure 4.3C are validated using observations of PTK7$^+$ naive CD4$^+$ T cells in healthy, non-thymectomised individuals of different ages, as measured by Haines et al. [3]. According to the simple survival model defined previously, the frequency of circulating PTK7$^+$ naive CD4$^+$ T cells with age is simply a convolution of thymic production and the survivorship of PTK7$^+$ naive CD4$^+$ T cells (equation (4.1)). It is assumed, parsimoniously, that survivorship of thymic emigrants within the PTK7$^+$ naive CD4$^+$ T cell compartment is independent of the age of host at time of export. Quantitative estimates of daily thymic export, derived in chapter 3, are combined with the feasible survivorship functions to reconstruct the change in circulating PTK7$^+$ naive CD4$^+$ T cell numbers with age (Figure 4.3D). The model simulations are able to capture the observed change in PTK7$^+$ naive CD4$^+$ T cell numbers with age [3].

Finally, a least-square optimisation is used to identify the survivorship function that lies within the limits determined by data from thymectomised patients and best describes the change in PTK7$^+$ naive CD4$^+$ T cell numbers in healthy individuals with age (Table 4.1). The derived function corresponds to an expected residency time of $\sim 23.3$ days within the PTK7$^+$ naive CD4$^+$ T cell population (solid black curve in Figure 4.3C). This reflects a situation where 95% of thymic emigrants will transit through the PTK7$^+$ stage within 25 days, although the key to explaining the persistence of PTK7$^+$ T cells post-thymectomy is that the distribution is inherently heavy-tailed and $\sim 0.4\%$ of cells will persist for greater than a year. Biologically, this might reflect a progressive decline in the expression of co-stimulatory or signalling molecules with increasing time spent in the periphery that reduces the probability a PTK7$^+$ T cell will mature into the PTK7$^-$ naive pool. Alternatively, the long-lived fraction of PTK7$^+$ naive CD4$^+$ T cells may represent a subpopulation of cells that are intrinsically less responsive to maturation stimuli from time of export, perhaps related to heterogeneity in T cell receptor specificity.
Figure 4.3: A,B: Feasible decline in PTK7+ naive CD4+ T cells post-thymectomy is limited by clinical observations by Haines & colleagues [3] (filled circles) and measurement error of ± 20 cells /µl (black lines) in individuals aged 2 and 14, respectively; C: Feasible PTK7 survivorship functions corresponding to observed decay of PTK7+ naive CD4+ T cells; D: PTK7+ naive CD4+ T cell numbers observed in non-thymectomised, healthy individuals (filled circles) by Haines & colleagues [3] compared to PTK7+ naive CD4+ T cell numbers simulated using equation (4.1) in combination with independent estimates of thymic export (chapter 3) and feasible survivorship functions. Solid black line represents the simulation that best describes the observations in healthy individuals and corresponds to the survivorship function (black line) in Figure 4.3C.
### Table 4.1: Observed frequency of PTK7$^+$ naive CD4$^+$ T cells (cells/µl) in healthy individuals aged 0 to 60 years. Data from Haines et al. [3]

<table>
<thead>
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<th>Subject ID</th>
<th>Age (years)</th>
<th>PTK7$^+$ Naive CD4$^+$ T cells (cells/µl)</th>
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</thead>
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<tr>
<td>C01</td>
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<td>317</td>
</tr>
<tr>
<td>C02</td>
<td>5</td>
<td>180</td>
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<td>C04</td>
<td>5</td>
<td>128</td>
</tr>
<tr>
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<td>64.</td>
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<tr>
<td>C06</td>
<td>14</td>
<td>160</td>
</tr>
<tr>
<td>C07</td>
<td>14</td>
<td>44.</td>
</tr>
<tr>
<td>C08</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>C09</td>
<td>25</td>
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</tr>
<tr>
<td>C16</td>
<td>52</td>
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</table>

PTK7$^+$ naive CD4$^+$ T cells do not necessarily represent recently emigrated cells

In this section, the implications of a heavy-tailed distribution of residency times combined with cumulative thymic export over the lifespan of an individual are explored. The survivorship function that is best able to reproduce quantitative changes in PTK7$^+$ naive CD4$^+$ T cell numbers with age (Figure 4.3C) is used together with the model to reconstruct the PTK7$^+$ T cell population and track the fate of cells according to their post-thymic age (described in more detail in Appendix 4.5.1). In agreement with observed cell concentrations in peripheral blood [3], the model predicts that total body numbers of PTK7$^+$ naive CD4$^+$ T cells decline continuously from a peak at age 1 (Figure 4.4A).

The number of ‘most recent’ thymic emigrants, ie. the number of PTK7$^+$ naive CD4$^+$ T cells that have spent less than 3 months in the periphery, appear to peak at age 1 years and then decline in a bi-phasic manner to age 60 years, in line with diminishing thymic export (see Figure 3.4C). In contrast, the total number of veteran PTK7$^+$ naive CD4$^+$ T cells (defined as cells that have spent
more than a year in the periphery) increases to age 8 years and then declines ~15-fold to age 60 (Figure 4.4A). As a result, the veteran cells represent an increasingly dominant fraction of the PTK7+ naive CD4+ T cell population with age. The percentage of veteran cells is predicted to increase from ~25% at age 2 years to ~85% at age 60 years. As a consequence, the average post-thymic age of peripheral PTK7+ naive CD4+ T cells is predicted to increase almost linearly from approximately 0 at birth to ~21 years at age 60; the average post-thymic age of a PTK7+ naive CD4+ T cells in a young adult is predicted to be ~6.5 years (Figure 4.4C). The dramatic increase in the average post-thymic age of cells with aging described here occurs in the absence of any change in the residency time of newly-exported emigrants or change in environmental stimuli and is a direct result of the preferential accumulation of ‘long-lived’ cells from within each cohort of thymic emigrants. In appendix Figure 4.8 an alternative lognormal distribution is used to describe the change in PTK7+ T cell numbers and it is found that the increase in post-thymic age is robust to the heavy-tailed distribution chosen to fit the data.

**Transition from the PTK7+ to the PTK7- naive compartment is almost exclusively associated with the most-recently emigrated T cells**

The average rate at which cells leave the PTK7+ naive CD4+ T cell population is estimated as follows:

$$\delta(t) = \frac{\int_0^t \theta(a)f_a(t-a)da}{X(t)}$$

(4.11)

where $f_a(x)$ represents the fraction of cells exported at age $a$ that will be lost from the PTK7+ population on day $x$ post-export, such that $F_a(t) = \int_0^t f_a(x)dx$; and $X(t)$ is the size of the PTK7+ naive CD4+ T cell population at age $t$, as before. The expected distribution of PTK7 residency times identified in Figure 4.3C predicts that in accord with the increase in average time spent in the periphery (Figure 4.4C), the average rate of conversion from PTK7+ to PTK7−
4.3 Results

Figure 4.4: Implications of a heavy-tailed distribution of PTK7$^+$ naive CD4$^+$ T cell residency times for natural aging. Predicted peripheral PTK7$^+$ naive CD4$^+$ T cells, in terms of total cell numbers (A) and percentage of cells (B) within each post-thymic age category, is simulated using the best-fit PTK7$^+$ naive CD4$^+$ T cell survivorship function (described in Figure 4.3C) and quantitative estimates of daily thymic export (described in Figure 3.4). C: Average post-thymic age of peripheral PTK7$^+$ naive CD4$^+$ T cells as a function of age; D: Predicted per-cell rate of transition and post-thymic age of PTK7$^+$ T cells lost to PTK7$^-$ naive T cell population.
4.3 Results

drops ∼6-fold from ∼10% to ∼1.6% of PTK7⁺ T cells per day from age 1 to 60 years (Figure 4.4D).

The post-thymic age of cells lost from the PTK7⁺ naive CD4⁺ T cell pool was also calculated. Despite the dramatic shift in the post-thymic age of PTK7⁺ naive CD4⁺ T cells in aging individuals (Figure 4.4B), the post-thymic age of cells transitioning from the PTK7⁺ to PTK7⁻ pool appears to be independent of age, that is, greater than 99% of cells leaving the PTK7⁺ naive CD4⁺ T cell compartment are predicted to have spent less than 1 month in the periphery, even in a 60 year old individual (Figure 4.4D).

Thymectomy results in an accelerated increase in the average post-thymic age of PTK7⁺ naive CD4⁺ T cells

Thymectomy is simulated in typical individuals aged 2 and 14 years according to the variation model (equation 4.2), allowing conservatively for the persistence of 5% of original thymic production (Figure 4.5A & B). In appendix Figure 4.9 it is shown that the post-thymectomy dynamics are fairly robust to estimates of residual thymic production varying from 0 to 20%. The model allows us to investigate the implications of thymectomy, combined with natural variation in residency times, on the composition of the residual PTK7⁺ naive CD4⁺ T cell population in a way that is difficult to observe experimentally. The best-fit distribution of PTK7⁺ residency times suggests that the majority of cells will transition into the more mature population within weeks of export. This transitional component of the PTK7⁺ population will be rapidly depleted as it fails to be replaced by de novo production of T cells by the thymus while a fraction of veteran PTK7⁺ T cells will continue to persist.

In a non-thymectomised 2 year old, the most-recently emigrated T cells comprise ∼70% of the PTK7⁺ compartment and since the model predicts that these cells are preferentially depleted following thymectomy, the average post-thymic age of PTK7⁺ naive CD4⁺ T cells increases 3-fold within 2 months of thymectomy. A similar process occurs in a 14 year old and the average post-
4.3 Results

Thymic age of PTK7\(^+\) naive CD4\(^+\) T cells is estimated to increase from \(\sim6.3\) to \(\sim8.3\) years within 2 months of thymectomy mirroring an accelerated form of the increase in post-thymic age of cells that is predicted to coincide with natural aging. The variation model suggests that simply observing the change in T cell numbers, even within individual T cell subsets, may underestimate the impact of thymectomy. The post-thymic age of PTK7\(^+\) naive CD4\(^+\) T cells in thymectomised children appears to more closely resemble that of older individuals than healthy, age-matched controls.

This is in contrast to the predictions of the homogeneity model, where the probability of transition, from PTK7\(^+\) to PTK7\(^-\) naive T cells, is homogeneous across the entire PTK7\(^+\) population. In Figure 4.6, the homogeneity model is used to describe the post-thymectomy dynamics simulated in Figure 4.5. Using equation 4.10, the average rate of PTK7\(^+\) naive CD4\(^+\) T cell loss is predicted to decline rapidly following thymectomy (Figure 4.6A & B). Unlike the variation model however, this model predicts that all cells are lost at the same rate and a preferential loss of most-recently emigrated cells post-thymectomy is not observed. In Figure 4.6C & D the post-thymic age of cells is assumed to be identical to that simulated under the variation model until time of thymectomy, allowing the way in which thymectomy differs under the two alternative models to be highlighted. Following thymectomy in the homogeneous model, the only observed change in the composition of the residual PTK7\(^+\) naive CD4\(^+\) T cell population arises from the natural aging of cells. As a result, the homogeneity model predicts a slower linear increase in average post-thymic of PTK7\(^+\) naive CD4\(^+\) T cells, that is quite distinct from the sudden jump in average post-thymic age that is predicted by the variation model.
Figure 4.5: Predicted implications of thymectomy on the post-thymic age of PTK7+ naive CD4+ T cells when there exists inter-cellular variation in the rate of transition, from PTK7+ to PTK7− naive CD4+ T cells. A & B: Absolute decline in total number and post-thymic age of PTK7+ naive CD4+ T cells; C & D: Average post-thymic age of the persistent PTK7+ naive CD4+ T cell population following thymectomy in a typical 2 and 14 year old, respectively; E: Predicted size of persistent PTK7+ naive CD4+ T cell pool following thymectomy at different ages, as a percentage of expected cell numbers in age-matched non-thymectomised individuals.
Figure 4.6: Predicted implications of thymectomy on the post-thymic age of PTK7\(^+\) naive CD4\(^+\) T cells when rate of transition, from PTK7\(^+\) to PTK7\(^-\) naive CD4\(^+\) T cells, is homogeneous across PTK7\(^+\) naive CD4\(^+\) T cell population. A & B: Average per-cell rate of transition from PTK7\(^+\) to PTK7\(^-\) naive CD4\(^+\) T cells; C & D: Decline in total number and post-thymic age of PTK7\(^+\) naive CD4\(^+\) T cells; E & F: Average post-thymic age of residual PTK7\(^+\) naive CD4\(^+\) T cell population following thymectomy in a typical 2 and 14 year old, respectively.
Variation in residency times naturally explains the persistence of a larger population of PTK7$^+$ naive CD4$^+$ T cells following thymectomy in older individuals through the accumulation of persistent veteran PTK7$^+$ T cells with age.

The experimental data shows and the model predicts that PTK7$^+$ naive CD4$^+$ T cell numbers approach a quasi-set point within 250 days of thymectomy. The size of the PTK7$^+$ naive CD4$^+$ T cell pool at day 250 post-thymectomy was estimated and used to explore the new set point that would be reached following thymectomy in individuals of different ages under the variation model. This provides a testable prediction of our hypothesis where data from additional patients could be used to verify the model. As before thymectomy was simulated in typical individuals aged 0 to 60 years by assuming that residual thymic production would be equivalent to 5% of original output. As expected, the number PTK7$^+$ T cells on day 250 after thymectomy, as a fraction of expected cell numbers in healthy, age-matched controls, increases continuously with age. The steepest increase was observed from birth to age 10 years. Approximately 15% of PTK7$^+$ naive CD4$^+$ T cells are expected to persist following thymectomy at age 1 compared to $\sim$70% by age 10 years. Persistence of PTK7$^+$ naive CD4$^+$ T cells to day 250 does not increase much from age 10 to 60 years (from 70% to 82%). This compares to the observed persistence of $\sim$15% and $\sim$45% of PTK7$^+$ naive CD4$^+$ T cells in thymectomised subjects aged 2 and 14 years, respectively [3].

It is not intuitively obvious that thymectomy in early childhood should result in a smaller long-lived residual PTK7$^+$ naive CD4$^+$ T cell population given that the ‘starting’ population is largest in these same individuals. The variation model predicts, however, that the PTK7$^+$ naive CD4$^+$ T cell population in younger individuals predominantly consists of transitional cells with a high rate of conversion into the PTK7$^-$ naive CD4$^+$ T cell pool that are rapidly lost following the suppression of thymic output. With age, the model predicts the accumulation of more persistent cells and hence a smaller drop in PTK7$^+$ naive
4.4 Discussion

The immune system as a whole is fairly resilient to thymectomy. Sempowski and colleagues [112] showed that the concentration of naive CD4\(^+\) T cells did not fall significantly following treatment of myasthenia gravis by thymectomy and the concentration of memory CD4\(^+\) T cells was actually observed to increase. The response of the naive T cell population to a drop in thymic output is likely to represent a complex product of a change in cell survival, proliferation, differentiation and death. In this chapter, the changes that occur within the more-recently emigrated PTK7\(^+\) naive CD4\(^+\) T cell subset are explored. The kinetics are easier to interpret as compared to the naive T cell population since it is thought that both proliferation and differentiation lead to the loss of cells from the PTK7\(^+\) naive CD4\(^+\) T cell subset. Haines and colleagues [3] observed that, following an initial drop in cell numbers, subjects were able to maintain a fairly stable residual population of PTK7\(^+\) naive CD4\(^+\) T cells for up to 6 months post-thymectomy. In this work, the underlying homeostatic mechanisms that might explain the post-thymectomy kinetics were explored.

It was argued that incomplete thymectomy alone is unable to account for the persistent fraction of PTK7\(^+\) naive CD4\(^+\) T cells post-thymectomy (Figure 4.2). Instead it was shown that the average rate at which cells are lost from the PTK7\(^+\) naive CD4\(^+\) T cell population must decline post-thymectomy.

One possible explanation for the increased persistence of PTK7\(^+\) naive CD4\(^+\) T cells might be a time dependent rate of transition, from PTK7\(^+\) to PTK7\(^-\) naive CD4\(^+\) T cells, that is assumed to be homogeneous across the population but declines in response to thymectomy. This might reflect a system where the persistence of PTK7\(^+\) T cells is related to the size of the PTK7\(^+\) pool. For example, if persistence of PTK7 expression on naive T cells was dependent on some resource, perhaps cytokine stimulation, then competition for this stimuli would lead to a low rate of PTK7\(^+\) T cell loss when PTK7\(^+\) T cell densities were
low and vice-versa.

Alternatively, as proposed here, natural inter-cellular variation in the rate of transition could also explain the residual population. Under this hypothesis, the residual PTK7$^+$ population is explained by the endurance of naturally more-persistent, veteran, PTK7$^+$ naive CD4$^+$ T cells. The bi-phasic decline in PTK7$^+$ naive CD4$^+$ T cells is an outcome of pre-existing heterogeneity and the decline in average per-cell rate of transition simply reflects the preferential loss of PTK7$^+$ T cells with intrinsically shorter residency times.

Both models can explain the long term survival of PTK7$^+$ naive CD4$^+$ T cells post-thymectomy. The mathematical analysis described here, however, identifies that the two models have significantly different implications for our understanding of the consequences of thymectomy and aging. The inter-cellular variation model is characterised by an accelerated increase in the post-thymic age of PTK7$^+$ naive CD4$^+$ T cells, or persistence of veteran cells, following thymectomy, whereas, the homogeneity model is associated with a much slower increase in the average post-thymic age of PTK7$^+$ naive CD4$^+$ T cells at a rate that simply mirrors the natural aging of cells.

In reality, both natural inter-cellular variation and global changes to the rate of PTK7$^+$ T cell loss might play a role in the PTK7$^+$ naive CD4$^+$ T cell response to thymectomy. An experimental approach that was able to measure the post-thymic age of residual cells following thymectomy might allow us to better determine the relative contribution of inter-cellular variation to T cell homeostasis. In this work, the role of peripheral expansion of PTK7$^+$ naive CD4$^+$ T cells is ignored, however, in appendix 4.5.2 the model is extended to include a constant rate of division and the results are found to be robust to background division within the PTK7$^+$ pool. Furthermore, experimental studies measuring division within the PTK7$^+$ naive CD4$^+$ T cell population post-thymectomy could quite easily allow the role of peripheral expansion to be determined.
4.4 Discussion

Do PTK7\(^+\) naive CD4\(^+\) T cells represent recent thymic emigrants?

Under the inter-cellular variation model, a heavy-tailed distribution of residency times is best able to describe the post-thymectomy kinetics and the change in PTK7\(^+\) naive CD4\(^+\) T cell numbers with age. An inherent consequence of a heavy-tailed distribution is that, more-persistent, veteran PTK7\(^+\) T cells will preferentially accumulate in an aging host resulting in a progressive increase in the average post-thymic age of PTK7\(^+\) naive CD4\(^+\) T cells. Despite the relatively short expected residency time of thymic emigrants within the PTK7\(^+\) T cell population of \(~23\) days, the accrual of a small persistent fraction of cells from within each cohort of thymic emigrants over a number of decades can lead to an average post-thymic age of PTK7\(^+\) naive CD4\(^+\) T cells of \(~10\) years in a young adult. This suggests that although the PTK7\(^+\) naive CD4\(^+\) T cell population contains recent emigrated T cells, not all PTK7\(^+\) cells are necessarily recent emigrants. Although there are few experimental studies exploring the lifespan of recent thymic emigrants, or more specifically PTK7\(^+\) naive CD4\(^+\) T cells, this is consistent with the idea that the naive CD4\(^+\) T cell population in older individuals is maintained by longer-lived cells [165–168]. Furthermore, the accrual of persistent veteran T cells with age naturally explains the persistence of a larger fraction of PTK7\(^+\) naive CD4\(^+\) T cells following thymectomy in older individuals.

Thymectomy and premature aging of the T cell pool

Studies have suggest that the altered size of naive T cell subpopulations, for example percentage of CD31\(^+\) naive T cells, in thymectomised individuals resemble observations in older subjects [113, 114]. However, it is shown here under the variation model that even changes to the size of naive T cell subpopulations may underestimate the true impact of thymectomy. Murine studies have shown that naive CD4\(^+\) T cells derived from middle-aged thymectomised mice are intrinsically longer-lived when adoptively transferred into younger hosts, and more-closely resembled T cells from older donors, than T cells de-
4.4 Discussion

Derived from non-thymectomised mice [168]. Consistent with this observation, the theoretical model presented here shows an accelerated increase in the average post-thymic age of residual PTK7+ naive CD4+ T cells, or equivalently a dominance of veteran cells, in a thymectomised individual that is more typical of aged individuals. Furthermore, a recent vaccine study reported delayed IgG production in response to tick-borne encephalitis virus vaccine in both thymectomised children and elderly subjects [115].

The mechanisms that lead to age-associated changes in T cell behaviour are not well understood. Adoptive transfer of young and aged bone marrow cells into young and aged murine hosts indicate that both intrinsic changes in T cell progenitors and defects in the aged environment in which cells develop are partially responsible for the age-associated defects in function of recently emigrated T cells [169]. Furthermore, depletion of naive CD4+ T cells followed by reconstitution by aged bone marrow seems to repair age-related dysfunction and newly produced T cells are found to be functionally similar to cells from young mice [166, 169]. Together these observations suggest that aging of the T cell population is driven by a complex product of extrinsic factors, such as cytokine availability and size of the overall naive and memory compartments, and intrinsic factors, perhaps related to the changing expression of co-stimulatory molecules, T cell receptors and other signalling proteins with age.

A number of recent murine studies suggest that increased longevity of naive CD4+ T cells facilitates the accumulation of functional defects [166, 168, 170]. If we conjecture that the age of a cell is associated with function, then the increase in average post-thymic age of cells as a result of the variation model described here, suggests that inter-cellular variation might represent one more important component of the aging process.
4.4 Discussion

Does post-thymic positive selection shape the PTK7^− naive population?

Theoretically, there are a number of possible biological explanations for a heavy-tailed distribution of PTK7 residency times, or equivalently a decline in average rate at which cells transition from PTK7^+ to PTK7^− naive CD4^+ T cells with increasing time spent in the periphery. The intrinsic ability of cells to transition into the PTK7^− population may diminish with time if, for example, necessary co-stimulatory or signalling molecules highly expressed at time of exit from the thymus slowly diminish with time spent in the periphery. Alternatively, variation in rate of transition from PTK7^+ to PTK7^− naive T cells might reflect natural heterogeneity within each cohort of emigrants, where the residency time of a thymic emigrant in the PTK7^+ state might be determined by the strength of TCR affinity, co-receptor density, or simply stochastic variation in expression of signalling proteins.

A corollary to the latter model is that the recruitment of peripheral PTK7^+ naive CD4^+ T cells into the mature PTK7^− naive T cell population involves an element of post-thymic positive selection. The analysis presented here suggests that despite a dramatic increase in the average post-thymic age of PTK7^+ naive CD4^+ T cells in older individuals, the post-thymic age of cells entering the PTK7^− population is almost exclusively less than 3 months. If it assumed that the residency time of a PTK7^+ naive CD4^+ T cell is linked to the ability of the cell to respond to some maturation signals, then the 'shorter-lived' PTK7^+ cells will represent the most responsive among all PTK7^+ thymic emigrants. The preferential incorporation of the more-responsive thymic emigrants into the PTK7^− naive CD4^+ T cell population might explain why PTK7^− naive CD4^+ T cells are more responsive to αβ-TCR/CD3 and CD28 engagement than PTK7^+ naive CD4^+ T cells [3]. The differential responsiveness of resident naive T cells and RTEs is also observed mice [87, 171]. Further experimental studies exploring the transition from PTK7^+ to PTK7^− naive CD4^+ T cells will be important not only for determining how well PTK7 expression correlates to recent thymic emigration, but also for better understanding how new T cells are incorporated
Appendices

4.5.1 Modeling division within the PTK7$^+$ naive CD4$^+$ T cell population

The model of PTK7$^+$ naive CD4$^+$ T cell dynamics (equation 4.1) can be extended to allow for homeostatic division within the PTK7$^+$ naive CD4$^+$ T cell population. We introduce a new variable, $M(x)$, to represent the number of division events taking place within $x$ days of a PTK7$^+$ naive CD4$^+$ T cell leaving the thymus. The predicted size of the PTK7$^+$ naive CD4$^+$ T cell population at any given time becomes a function of thymic export, the lifespan of cells and the expected number of divisions:

$$X(t) = \frac{1}{\theta(t)} F_x(t-x) 2^{E[M(t-x)]} dx$$

where $E[M(x)]$ is the expected number of divisions occurring in $x$ days of exit from the thymus. In the absence of experimental data, we make the parsimonious assumption that the residency time of PTK7$^+$ naive CD4$^+$ T cell is independent of homeostatic division events.

Using the same approach described in section 4.2, we compare equation 4.12 to a corresponding expression for the decay of PTK7$^+$ naive CD4$^+$ T cell following thymectomy, allowing for division, to derive an expression for the residency function of peripheral PTK7$^+$ naive CD4$^+$ T cells :

$$F_{t_0}(i) \approx 1 \frac{dX^*(i \text{ days post thymectomy})}{dt}$$

where, in the absence of experimental data we assume that the probability of a PTK7$^+$ naive CD4$^+$ T cell dividing on any given day is constant, $p$ and independent of time since export. Hence the expected number of divisions in $i$ days becomes $qi$. 
Observing the change in PTK7$^+$ naive CD4$^+$ T cells following thymectomy alone does not allow us to disentangle the effect of cell persistence and expansion. So we let the average rate of division within the PTK7$^+$ naive CD4$^+$ T cell pool ($q$) equal 0, 0.01, 0.10 and 0.50 (day$^{-1}$). An arbitrary decay of PTK7$^+$ T cells post-thymectomy is used to explore the impact of the different rates of background division on the underlying rate of loss of PTK7$^+$ naive CD4$^+$ T cells (Figure 4.7). It can be shown that the per-cell rate of loss increases by a constant amount, $q \log[2]$, to compensate for a constant rate of division of $q$ (day$^{-1}$) within the PTK7$^+$ naive CD4$^+$ T cell population.

Despite the unknown rate of division, the composite function of survival and expansion of PTK7$^+$ naive CD4$^+$ T cells in equation 4.12, $F_x(t - x)2^{E[M(t-x)]}$, is identical to the survivorship computed in the simpler, no-division model. So although the relative contribution of expansion and survival can not be quantified, the net survivorship can be estimated and hence the observed shift in post-thymic age of PTK7$^+$ naive CD4$^+$ T cells will be robust to a constant rate of background division.

**4.5.2 Estimating Post-thymic Age of the PTK7$^+$ naive CD4$^+$ T cells**

Using the predicted residency times of PTK7$^+$ naive CD4$^+$ T cells following thymic export, $F_x(t)$, the number of PTK7$^+$ naive CD4$^+$ T cells of a certain post-thymic age, in an individual aged $t$, can be estimated as follows:

$$N_h(t) = \int_{t-h}^{t} \theta(x)F_x(t - x)dx \quad (4.14)$$

where $\theta(x)$ is the rate at which PTK7$^+$ naive CD4$^+$ T cells are exported by the thymus and $N_h(t)$ represents the number of PTK7$^+$ naive CD4$^+$ T cells in an individual aged $t$ exported within the past $h$ days ($h < t$).
Figure 4.7: Interpreting the decay of PTK7+ naive CD4+ T cells post-thymectomy using a model of division within the PTK7+ naive CD4+ T cell population. Let the rate of division (p) equal 0 (solid line), 0.01 (dotted line), 0.1 (short dashed line) and 0.5 (large dashed line) (day$^{-1}$) and explore the predicted residency times. A: Arbitrary decline in PTK7+ naive CD4+ T cell numbers post-thymectomy in a subject aged 14; B: Estimated survivorship function of PTK7+ naive CD4+ T cells corresponding to PTK7+ T cell decay in A, where the rate of division (p) is set to 0 (solid line), 0.01 (dotted line), 0.1 (short dashed line) and 0.5 (large dashed line) (day$^{-1}$); C: Corresponding estimate for instantaneous per-cell rate of loss from the PTK7+ naive CD4+ T cell population as a function of time since export.
Figure 4.8: Using a lognormal distribution to describe the survivorship function of PTK7+ naive CD4+ T cells

A: Using the same approach as in Figure 4.3, a lognormal PTK7+ T cell survivorship function is identified that best describes PTK7+ naive CD4+ T cell numbers in healthy individuals with age but is limited so that it also lies within the feasible range to describe post-thymectomy dynamics; B: PTK7+ naive CD4+ T cell numbers observed in non-thymectomised, healthy individuals (filled circles) by Haines & colleagues [3] compared to PTK7+ naive CD4+ T cell numbers simulated using best-fit lognormal survivorship function; C: Predicted post-thymic age of the peripheral PTK7+ naive CD4+ T cells population with age (absolute number of cells); D: Relative size of each post-thymic age category; E: Average post-thymic age of peripheral PTK7+ naive CD4+ T cells as a function of age.
Figure 4.9: **Modelling variation in residual thymic production.** In this study the implications of thymectomy are explored by assuming that ~5% of thymic production continues after thymectomy. Here we explore the robustness of the model predictions by varying residual thymic production from 0 to 20%. **A:** Predicted decline in total number of PTK7+ naive CD4+ T cells and corresponding post-thymic age of cells; **C & D:** Average post-thymic age of residual PTK7+ naive CD4+ T cells following thymectomy in a typical 2 year old where residual thymic production is assumed to be 0% and 20%, respectively.
Chapter 5

Conclusion

T cell homeostasis enables the immune system to maintain a stable naive T cell population throughout adulthood. Better understanding of the natural aging process and the ability of the immune system to adapt to declining thymic production provides further insight into situations where T cell homeostasis is disturbed leading to T cell depletion, such as HIV infection and intentional radiation- or drug-induced immuno-depletion.

Aging and T cell homeostasis

Aging is associated with an increase in infection, morbidity and decline in efficacy of vaccines [172]. The mechanisms that lead to age-associated changes in T cell function are not well understood. The ability to maintain T cell numbers and diversity with age is vital to the function of T cells in the adaptive immune system. New specificities are added to the peripheral T cell repertoire through the incorporation of newly formed lymphocytes from the thymus, while peripheral division has the ability to modulate the frequency of specific T cell receptors. Knowledge of the relative contributions of thymic production and peripheral expansion is important to better understanding the establishment of the T cell repertoire. The aim of this thesis was to investigate the T cell kinetics that regulate development, maintenance and subsequent aging of the naive T cell compartment.

In this work, the relative contribution of thymic production and peripheral ex-
expansion was shown to be approximately constant from birth to age 20 years. The daily contribution of peripheral expansion to the naive T cell population was found to be approximately 2-fold greater than that of thymic output. This is in accord with the hypothesis that thymic selection and export may not be the primary force in shaping the T cell repertoire as previously thought.

Past studies of naive T cell dynamics assumed that rates of both proliferation and loss are dependent on the size of the naive T cell population [53, 126]. In chapter 2, the average residency time of naive CD4+ T cells was shown to increase with age suggesting that cells become longer-lived as the age of the host increases, despite an increase in the absolute naive CD4+ T cell population. In chapter 4, a model of natural inter-cellular variation in the residency times of PTK7+ naive T cells was shown to be associated with an increase in the average post-thymic age of PTK7+ naive CD4+ T cells in aging individuals. This model might be extended in the future to the entire naive T cell population to provide a possible mechanism for explaining the density-independent decline in rate of loss of naive T cells with age. The model is characterised by an accumulation of more-persistent T cells with age and might have serious consequences for clonal diversity of T cells in an aging individual if residency time was linked to TCR affinity. The preferential accumulation of T cells associated with particular TCR chains with age might contribute to the contraction of T cell population diversity observed in elderly subjects [70].

In estimating the average rates of naive CD4+ T cell production and loss, it was assumed that the population is homogeneous. It is known that the T cell population can be divided into sub-populations of more, or less, recently emigrated cells, determined by expression of markers such as PTK7 and CD31, where the relative size of each subpopulation changes with age. An extension to this work could look more carefully at whether turnover occurs at the same rate within different naive CD4+ T cell subpopulations.
T cell depletion and T cell homeostasis

HIV infection is associated with a progressive depletion of CD4\(^+\) T cells. At present there is no vaccine or cure for HIV but highly active anti-retroviral therapy (HAART) is used to control the virus and delay the onset of acquired immunodeficiency (AIDS). There were over 33 million people living with HIV at the end of 2008, of which 2.1 million were children. Yet the nature of T cell depletion associated with HIV and its underlying causes remain controversial. The natural progression of HIV is typically characterised by a steep initial drop in CD4\(^+\) T cell numbers during the first few weeks of infection followed by an asymptomatic chronic period during which CD4\(^+\) T cell counts decline gradually over a number of years. In addition, disease progression has been shown to be closely associated with increased division and activation of both CD4\(^+\) and CD8\(^+\) naive and memory T cells [59, 173–175]. The mechanisms by which increased CD4\(^+\) T cell proliferation is associated with a decline in CD4\(^+\) T cell numbers are not well understood and there exists significant inter-patient variation in the rate of disease progression. In particular, studies have shown that 20-30\% of vertically-infected infants will develop AIDS within the first year of life, while the average asymptomatic period in adults lasts \(\sim\)10 years [176,177]. It is not altogether surprising that there exists such differences between children and adults since T cell depletion is driven by an imbalance between rates of cell production and loss. The CD4\(^+\) T cell population in healthy adults is known to be relatively stable, whereas the number, naive and memory components, and thymic production of CD4\(^+\) T cells is known to change throughout childhood.

A number of studies have suggested that thymic production and peripheral proliferation of naive CD4\(^+\) T cells declines with age [1,2,8,52,70]. In this thesis, it was shown that the predicted evolution of both thymic production and peripheral expansion can be divided into at least three distinct age-related stages: (a) increase in T cell production from birth to age 1 year; (b) rapid decline until \(\sim\) age 8 years; and (c) gradual decline into adulthood. The shift towards a naturally less dynamics population with age is consistent with slower natural
disease progression in adults since productive HIV infection predominantly occurs in activated T cells. The increasing lifespan of naive T cells with age may also play a role in HIV latency and the accumulation of virus in resting cells.

Further work could quite feasibly use a similar mathematical approach to carefully re-evaluate the change in absolute naive CD4+ T cell numbers, TRECs and Ki67 expression in HIV infection in individuals of different ages to quantify thymic production. The impact of HIV and subsequent HAART on thymic production remains unclear [2, 153] although it is important for our understanding of the consequences of HIV for repertoire diversity as well as T cell counts. Interestingly, despite a decline in the concentration of cells, the absolute number of naive CD4+ T cells continues to increase with age in HIV-infected children, although at a slower rate than that expected for uninfected children, whereas adult infection is invariably linked to a decline in absolute CD4+ T cell counts [2]. The observations highlight the fact that HIV disease progression is determined by subtle perturbation in the rate of cell production and loss and mathematical models could play an important role in the future in better understanding HIV progression and response to HAART.

Immune reconstitution and T cell homeostasis

Appreciating the capacity of the immune system to repopulate the T cell population is important for predicting the outcome of HAART treatment as well as stem cell and thymic transplants. Severe autoimmune diseases such as systemic sclerosis, multiple sclerosis, systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis are increasingly treated by immunoablative chemotherapy followed by autologous hematopoietic stem cell transplantation (ASCT) [178]. The aim of such treatments is to remove inflammation-driving pathogenic cells from the immune system and reestablish immune tolerance. It is thought that the de novo generation of naive T cells is able to reset the immunological clock, however, the exact mechanism through which ASCT therapy works is not well understood. Long term analysis suggest that the fre-
quency of naive T cells in individuals that have undergone reconstitution exceeds that of healthy individuals, while the frequency of memory T cells is lower [179, 180]. Earlier observations, at 6 months post-therapy, suggest that the memory T cell compartment appears to initially outgrow the naive compartment [179, 180] in line with T cell reconstitution observed in HIV-infected adults undergoing HAART [136, 181]. In contrast, reconstitution of the T cell compartment in HIV infected children is dominated by naive CD4⁺ T cells from time of treatment initiation [182]. The kinetics of reconstitution appear to vary with time since therapy initiation, age of host and T cell population size and are not well defined. It is thought that the initial period of reconstitution is associated with extensive peripheral expansion of the T cell compartment, suggesting that the relative contribution of thymic production and lymphoehma induced proliferation may determine the relative reconstitution of the naive and memory T cell compartments.

Declining thymic production with age is consistent with the limited reconstitution of the naive compartment in adults and the predicted bi-phasic decline in thymic production might suggest that there exists a threshold age beyond which it is impossible to rebuild the entire naive T cell pool to normal values. It would be interesting to explore the extent to which reconstitution mirrors the original development of the T cell population during childhood.

The model of inter-cellular variation in residency times, described in chapter 4, leads to the accumulation of long-lived naive T cells in aging hosts and might provide one explanation for the restorative effect and reconstruction of a juvenile-like T cell population that follows ASCT. Furthermore, the ability of adults undergoing ASCT, unlike HIV-infected adults undergoing HAART, to successfully repopulate the naive T cell compartment might be evidence of a reversion of thymic involution. Using a similar mathematical approach to that described in chapter 3, the role of thymic rebound might be elucidated by quantifying the contribution of de novo thymic production to the reconstituted T cell pool.
Thymectomy and T cell homeostasis

The thymus is commonly removed in children undergoing open heart surgery and thymectomy was traditionally thought not to have clinical consequences in infants as young as 6 months [107]. However, a number of recent studies suggest that thymectomy may lead to premature immunosenescence of the T cell pool [114, 115]. In chapter 4, inter-cellular variation is shown to result in an accelerated increase in post-thymic age of T cells following thymectomy. It is not unreasonable to assume that the residency time of a T cell might be related to its functional properties. The model suggests that simply measuring T cell counts may underestimate the consequences of thymectomy and that the procedure may lead to a contraction of the T cell repertoire. More detailed experimental observations in combination with mathematical analysis could allow the residency time of other naive (and memory) T cell subsets to be explored and may provide a new window into the process of thymectomy.

Memory T cell homeostasis

In this thesis, the kinetic processes that guide the development of the naive T cell compartment during childhood are quantified. A key aspect of naive T cell dynamics is the loss of cells through differentiation into the memory T cell compartment. In addition to foreign-antigen induced memory T cell creation, it is thought that decreased competition for homeostatic signals in lymphopenic conditions leads to increased proliferation and differentiation of naive T cells into memory T cells [71]. The naive compartment is found to become progressively less dynamic from birth to adulthood, suggesting that the rate at which cells transition into the memory compartment might also declines with age. A natural extension of this work would involve exploring the kinetics of the memory CD4^+ T cells, perhaps looking at the extent to which the acquisition of new memory T cells related to the size of the memory and naive T cells compartments.
References


References


analysis of immune markers in a large population sample of healthy elderly individuals. Exp Gerontol 33: 593–600.


References


References


References


[175] Sousa, A. E., J. Carneiro, M. Meier-Schellersheim, Z. Grossman, and R. M. M. Victorino. 2002. CD4 T cell depletion is linked directly to im-


