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INVITED REVIEW

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Current best estimates for the average lifespans of mouse and human leukocytes: reviewing two decades of deuterium-labeling experiments

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Summary

Deuterium is a non-toxic, stable isotope that can safely be administered to humans and mice to study their cellular turnover rates in vivo. It is incorporated into newly synthesized DNA strands during cell division, without interference with the kinetics of cells, and the accumulation and loss of deuterium in the DNA of sorted (sub-)populations of leukocytes can be used to estimate their cellular production rates and lifespans. In the past two decades, this powerful technology has been used to estimate the turnover rates of various types of leukocytes. Although it is the most reliable technique currently available to study leukocyte turnover, there are remarkable differences between the cellular turnover rates estimated by some of these studies. We have recently established that part of this variation is due to (a) difficulties in estimating deuterium availability in some deuterium-labeling studies, and (b) assumptions made by the mathematical models employed to fit the data. Being aware of these two problems, we here aim to approach a consensus on the life expectancies of different types of T cells, B cells, monocytes, and neutrophils in mice and men. We address remaining outstanding problems whenever appropriate and discuss for which immune subpopulations we currently have too little information to draw firm conclusions about their turnover.

KEYWORDS

cell proliferation, cellular lifespans, deuterium labeling, immunological memory, modeling

1 | INTRODUCTION

The quantification of leukocyte dynamics is important for our understanding of the immune system in health and disease. There are many diseases—including, for example, autoimmune diseases and blood cell cancers—where the size of the leukocyte pool is changing, and where research is aiming to understand which factors cause or could repair the disturbed production or loss of specific leukocytes. For many types of leukocytes, however, even insights into the normal cellular dynamics were lacking until recently or are still heavily debated. The development of stable isotope-labeling techniques, using either deuterium-labeled glucose (D_2-glucose)[1,2] or deuterium-labeled water (D_2O),[3] has enabled the safe quantification of leukocyte turnover in humans in vivo, and has provided unprecedented insights into leukocyte dynamics in healthy individuals and patients. The earliest studies on the turnover of $CD4^+$ and $CD8^+$ T cells were performed around the turn of the millennium[4-7] and have paved the way for studies on the turnover of specifically defined leukocyte subsets, such as regulatory and stem-cell memory T cells, which have been performed more recently. Although deuterium labeling is the most reliable and the state-of-the-art technique to study leukocyte turnover, we have previously shown that published estimates of lymphocyte kinetics differ up to 10-fold between studies.[8] Thus,

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the current literature on in vivo deuterium labeling does not give unambiguous estimates. We here aim to reach consensus on the life expectancies of various leukocytes, to discuss strengths and pitfalls of different studies, and to discuss challenges for future research with this powerful technique. We will focus our review on leukocyte kinetics in healthy individuals.

Before reviewing the existing literature, it is important to provide some more detail on the different steps that are typically taken in deuterium-labeling studies. Both D_2-glucose and D_2O labeling studies rely on the principle that cells that undergo cell division incorporate deuterium into their DNA, which can be measured using a combination of gas chromatography (GC) and mass spectrometry (MS) analysis of the DNA in the isolated cell population. D_2-glucose is typically administered for short periods of time (hours or days), and since the pool of blood glucose is small and has a high rate of turnover, rapid up- and down-labeling can be achieved.[9] Although any part of the glucose molecule could be labeled, most studies have used 6,6-D_2-glucose. Due to intracellular dilution of label, enrichment levels in DNA maximally reach 60%-75% of the enrichment in plasma, which is accounted for by taking along a scaling factor in the range of 0.6-0.75.[2]

By contrast, D_2O is usually administered for several weeks. Body water turns over relatively slowly, so enrichment in the body fluids reaches its maximum and is washed out from the body much more slowly than D_2-glucose. Consequently, there is still considerable de novo DNA labeling after the discontinuation of label administration. The observed deuterium incorporation in the DNA of the cell population of interest is normalized to the (estimated) maximal level of deuterium incorporation in a cell population with rapid turnover, such as granulocytes, monocytes, or thymocytes.[3] This maximum enrichment attainable is determined by the level of D_2O in the body and by an amplification factor, previously referred to as *c*.[10] Since the deoxyribose moiety that is measured contains seven non-exchangeable hydrogen atoms, any of which is potentially replaced by deuterium, *c* is expected to be larger than one (and maximally seven) and typically lies in the range 3.5-5.2.[3,10,11]

Importantly, in both D_2-glucose and D_2O labeling studies, one needs to correct for the level of deuterium that is available in the body fluids, which is achieved by measuring deuterium enrichment of blood plasma or urine samples.[2,3] Since glucose turns over rapidly, the ratio of D_2-glucose over glucose in the serum is influenced by food consumption; the ratio decreases after food intake and increases during the night when food intake is low.[12] Participants in D_2-glucose labeling studies have to follow strict dietary restrictions on carbohydrate intake during labeling to limit these fluctuations. The availability of deuterium during D_2-glucose labeling is typically addressed by averaging, that is, by estimating the area under the curve of deuterium enrichment in plasma.[2] Since body water turns over much more slowly, the availability of deuterium in the body fluids during the labeling and de-labeling phase of D_2O experiments changes slowly and is typically described by a mathematical model fitted to the urine or plasma enrichment data.[10,13] This modeling also corrects for the continued de novo DNA labeling during the slow washout of D_2O from the body.

Our earlier review of lymphocyte dynamics revealed that 1 or 2 days of D_2-glucose labeling consistently yielded higher turnover rates than several weeks of D_2O labeling.[8] We have recently shown that—aside from the obvious reason that studies may differ in the markers used to sort cell populations—a large part of this variation is due to the problem of reliable normalization for deuterium availability.[12] In studies in which D_2-glucose is given overnight, deuterium availability tends to be underestimated, since plasma samples are typically not (or at least less frequently) taken during the night, when deuterium availability tends to be high. Such problems in normalization can have a major impact on the resulting estimates for leukocyte turnover.[12] Another problem with short-term labeling approaches (discussed in more detail below and in Ref. [8]) is that one can typically collect information during the down-labeling phase only. The earliest time point at which samples are taken is typically days after label administration has been stopped. The fraction of labeled cells at the peak can therefore not be measured directly and is instead estimated by back-extrapolation of the down-labeling curve to the moment at which label administration was stopped.[14] This is unfortunate, as the most informative part of the labeling curve is the up-labeling phase because it contains information about all cells in the population, not just the ones that have picked up the label.[14] Measurements during the up-labeling phase are, however, hard to obtain in short-term D_2-glucose labeling studies. For these reasons, when we review studies based on short-term overnight glucose labeling, we will not interpret the absolute values of the reported turnover rates, and only compare their relative values within each study. Importantly, while 1 or 2 days of labeling has its complexities and may be too short to reliably measure the turnover rates of populations of lymphocytes that are turning over at a time scale of months to years, the rapidity of glucose turnover makes D_2-glucose labeling the method of choice for estimating the turnover rates of rapidly turning over populations, like monocytes and neutrophils.[15-17]

Assuming that the appropriate labeling method has been chosen, the next challenge is to translate deuterium-labeling data into expected lifespans using mathematical models. When deuterium-labeling studies are performed in cell populations in steady state, that is, when cell numbers are constant, the average loss rate of cells should balance their average production rate. This is often referred to as the average turnover rate *d*, which naturally reveals the expected lifespan (or residence time) of cells, defined as $1/d$, as well as their half-life ($\ln[2]/d$). In several deuterium studies, the average turnover rate was called a "replacement" rate, which was derived from a simple exponential function.[18] Importantly, the up-labeling phase of a deuterium experiment contains most information about the average turnover rate of cells, while the decline in the fraction of labeled DNA during the down-labeling phase reflects the loss of cells that have recently picked up label.[14]

We have previously shown that in kinetically heterogeneous populations, that is, populations in which cells are turning over at different rates, the structure of the mathematical model can influence the interpretation of the data.[19,20] An example would be the labeling of the $CD4^+$ T cell pool, which is composed of naive T cells

and memory T cells, each with their own turnover rate (see Figure 1). The labeling kinetics of such kinetically heterogeneous cell populations have previously been described using the following model[14]:

$$l(t) = p/d^*(1-e^{d^*t}) \text{ during labeling, and}$$
$$l(t) = p/d^*(1-e^{-d^*t_{end}})e^{-d^*(t-t_{end})} \text{ during de-labeling} \quad (1a,b)$$

where $l(t)$ represents the fraction of labeled DNA, t_{end} defines the end of the labeling phase, p is the rate at which cells are produced, d^* is the rate at which labeled cells are lost, and where—for simplicity—we assume that the chance that a newly incorporated deoxyadenosine molecule is labeled is 1 during labeling and 0 during de-labeling. Using this model, one typically finds that $p < d^*$, because the cells with the highest turnover rates are overrepresented among the labeled cells.[14] We observed that when fitting the kinetic heterogeneity model of Equation (1) to labeling data from mice that were labeled for 1, 4 or 8 weeks, the estimated average turnover rate (p) became dependent on the length of the labeling period.[13] This may explain the observed positive correlation in the literature between the length of the labeling period and the estimated lifespan of cells.[8] The reason for this dependence is that in long-term labeling experiments, there is a risk that the enrichment of the fastest subpopulation starts to approach its plateau level, while the enrichment in the slower subpopulations is still increasing (see Figure 1). As a consequence, the kinetic heterogeneity model of Equation (1) will seek a compromise between the initial, steep increase (which represents the true average turnover rate of the cells under investigation) and the later, slower increase in label enrichment (representing the slower subpopulation). This will lead to an underestimation of the average turnover rate, especially when the labeling period is long[8,12,13]; see Figure 1. For self-renewing populations, that is, populations without a source, this problem was solved with the introduction of multi-exponential models that explicitly describe the kinetic heterogeneity[13,19-21]:

$$l_i(t) = a_i(1-e^{-d_i t}) \text{ during labeling, and}$$
$$l_i(t) = a_i(1-e^{-d_i t_{end}})e^{-d_i(t-t_{end})} \text{ during de-labeling,} \quad (2a,b)$$

where the population under investigation consists of n subpopulations, each with relative size a_i and with average turnover rate d_i. By increasing the number of subpopulations until the average turnover rate of the entire population converges into a final value, one can estimate the average turnover rate of a kinetically heterogeneous cell population (see Box 1 and Ref. [13] for further details).

In populations that are (partly) maintained by a source of novel cells from elsewhere, for example, new monocytes produced in the bone marrow or new naive T cells produced in the thymus, it becomes more challenging to interpret the data, because one needs to take the rate at which the source becomes labeled into account. Such labeling information about precursor cells outside the blood is often not available. In such cases, the information obtained from the labeling curves of cells in the blood may not always reflect the turnover of the cells in the blood and may be influenced—if not dominated—by the turnover of the precursor cells (see Box 1). Depending on the cell population under investigation, this may or may not pose a problem. An example where this does not pose a problem is the population of naive T cells, where the slow accrual and loss of label from cells in the blood truly represents the dynamics of naive T cells in the periphery, because their precursor cells in the thymus proliferate so rapidly that their label accrual and loss is no limiting factor. It may go wrong in the case of monocytes and neutrophils, where it is hard to distinguish

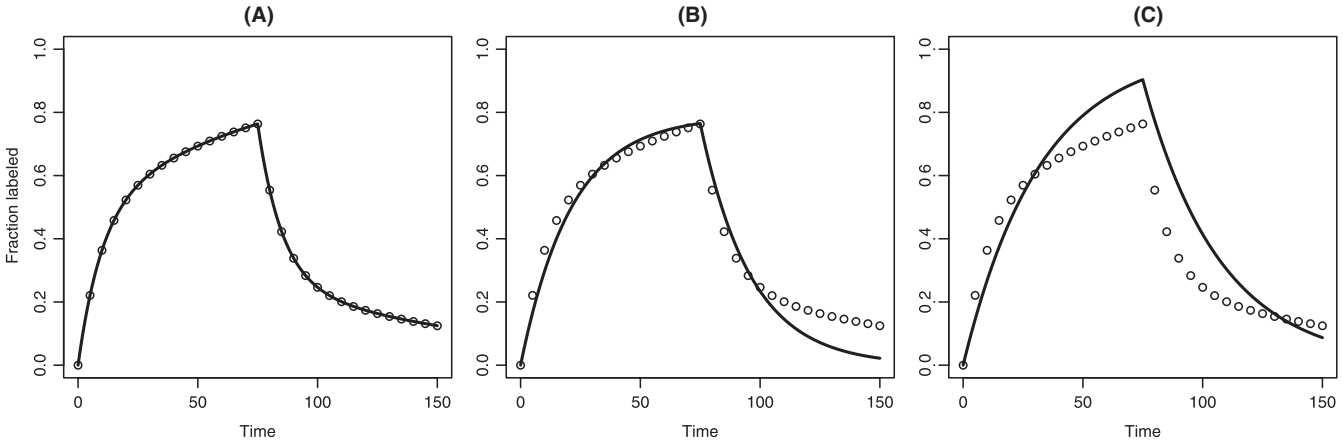

FIGURE 1 Fitting labeling data from a kinetically heterogeneous population. Each panel contains the same data, which were generated by solving the labeling equations of Equation (3), for a population consisting of two equally large subpopulations with average lifespans of 10 and 100 days, respectively (for a labeling phase of 75 days). One could think of labeling an "unsorted" $CD4^+$ T-cell population consisting of short-lived memory cells and long-lived naive T cells for 75 days. The data were fitted with Equation (3) and $n = 2$ in Panel (A), with the kinetic heterogeneity model of Equation (1) in Panel (B), and as a homogeneous compartment, that is, Equation (3) with $n = 1$, in Panel (C). The initial upslope reflecting the average turnover rate is underestimated in Panels (B) and (C) because the fitting procedure seeks a compromise between the steep initial upslope and the later slow phase when most of the cells in the rapid subpopulation have been labeled. Note that the kinetic heterogeneity model of Equation (1) in Panel (B) visually does a reasonable job at describing the upslope, but nevertheless underestimates the average turnover rate by one-third ($p = 0.037$, whereas $d = a_1d_1 + a_2d_2 = 0.055$; see Box 1). Additionally, it misses the long tail due to the long-lived cells in the downslope

Box 1 Mathematical models

Deuterium-labeling data have been fitted with a variety of models and unfortunately the turnover rates that are estimated from the data may crucially depend on the particular assumptions made by the mathematical model.[20] Ganusov et al[19] proposed a phenomenological model where the population of interest is divided into n subpopulations having different kinetics. Each subpopulation, i, comprises a fraction, a_i, of the whole population, and may have a source, s_i, a division rate, p_i, and a death rate, d_i. Dynamically, subpopulations are independent from each other, that is, they do not regulate each other and do not develop into each other. If all subpopulations are at steady state the source and division rate of each subpopulation has to balance the death rate of that subpopulation, and the model for the fraction of labeled cells in each subpopulation simplifies into:

$$l_i(t) = a_i(1 - e^{-d_i t}) \text{ when } t < t_{\text{end}}$$
$$l_i(t) = a_i(1 - e^{-d_i t_{end}})e^{-d_i(t - t_{end})} \text{ during de-labeling,} \quad (3a,b)$$

where t_{end} defines the end of the labeling phase, and—for simplicity—we assume that the chance that an incorporated deoxyadenosine molecule is labeled is 1 during labeling and 0 during de-labeling. The fraction of labeled DNA in the whole population, $l(t)$, is defined by the weighted sum of all subpopulations, that is, $l(t) = \Sigma\, a_i l_i$, and the average turnover, d, is defined by the weighted sum of all death rates, $d = \Sigma\, a_i d_i$. Since the model remains phenomenological, one should not attempt to overinterpret the individual fractions, a_i, or death rates, d_i. For instance, a population of memory T cells may be composed of many subpopulations each turning over at slightly different rates, and one can also derive a model without subpopulations by assuming a particular distribution of turnover rates in the population of interest.[19] Thus, the best procedure is to fit labeling data sequentially for an increasing number of subpopulations, that is, for various values of n, and halt when the *average* turnover rate, d, converges into a final value.[13,19] Finally, to use Equation (3) for fitting actual deuterium-labeling data, the model needs to be extended with a function describing the availability of deuterium,[13] which complicates the model mathematically, but not biologically.

The major advantage of this model is that it encompasses most earlier models. For a_i=n=1, the model has a single parameter, that is, the turnover rate d_1, and is identical to the single-exponential precursor-product relationship that is frequently used to model up-labeling data.[11] For n = 2, a_1<1, d_1>0, and d_2=0, the model has two parameters and describes a population composed of a fraction, a_1, of cells turning over at a rate d_1, while the remainder of the population, $a_2 = 1 - a_1$, has a turnover rate $d_2 = 0$, that is negligible at the time scale of the experiment. For these parameters, Equation (3) is identical to the frequently used single-exponential kinetic heterogeneity model (Equation (1)) proposed by Asquith et al,[14] and to the model used by Mohri et al,[7] which both predict an asymptote, $a_1 < 1$, in the labeling curve (see the derivation in De Boer & Perelson[20]). For n = 2, $a_1 < 1$, $d_1 > 0$, and $d_2 > 0$, the model contains three parameters, and not only describes a situation of kinetic heterogeneity due to the existence of two kinetically different subpopulations, but also describes earlier models for temporal heterogeneity[73] where recently divided cells may transiently experience a higher death rate than quiescent cells.[21] For all of these models, the average turnover rate, d, of the whole population is correctly defined (although the fractions, a_i, and individual turnover rates, d_i, may have very different interpretations given the underlying biology, and may be unreliable[20]).

For populations like monocytes and neutrophils that mature and divide as precursor cells in the bone marrow, and are later released into the peripheral blood as non-dividing circulating cells, B, we need another type of model.[12,15-17] A basic model for precursors, P, maturing and dividing in the bone marrow, and egressing from the bone marrow after a time delay, Δ, to form circulating cells, B, would be

$$P' = s - d_P P \text{ and}$$
$$B' = d_P P(t - \Delta) - d_B B \quad (4a,b)$$

where the prime denotes differentiation with respect to time, t, and s is the source of precursors from a hematopoietic progenitor compartment. It is also possible to write s=$d_P P$ to define a perfectly self-renewing precursor pool in the bone marrow.[15] Both versions of Equation (4) translate into a model similar to Equation (3) for the fraction of labeled DNA strands within the precursor pool in the bone marrow, that is,

$$l_P(t) = 1 - e^{-d_P t} \text{ when } t < t_{\text{end}}, \text{ and}$$
$$l_P(t) = (1 - e^{-d_P t_{end}})e^{-d_P(t - t_{end})} \text{ during de-labeling} \quad (5a,b)$$

The differential equation for the fraction of labeled DNA strands in the periphery becomes

$$l'_B = d_B(l_P(t - \Delta) - l_B), \quad (6)$$

which says that the enrichment in the blood approaches that in the bone marrow at a time scale d_B and after a time delay Δ.[12,15-17] This model can become quite complicated if the cells in the periphery consist of different phenotypes, like classical and non-classical monocytes.[16,17]

Box 1 (Continued)

A major problem with the model of Equations (5) and (6) appears when it is fitted to labeling data obtained from the circulation only, that is, when cells are sampled from the blood only, and not simultaneously from the bone marrow.[15,16,58,60] Since the model has two turnover rates, d_P and d_B, there are also two possible solutions to fit data that is measured in the periphery only (see Figure 2). For instance, finding a weekly turnover of label in the circulation can be due to a weekly turnover of cells in the peripheral blood, d_B = 1/7 per day, or due to much more rapid turnover of cells in the periphery, d_B = 1 per day, combined with a weekly turnover in the bone marrow, d_P = 1/7 per day (see Figure 2). There are two possible solutions to this problem.[15] First, one can simultaneously measure the fraction of labeled precursors in the bone marrow. Since this requires repeated measurements in a tissue that this difficult to access in volunteers, this is typically unfeasible in humans. Second, one may attempt to solve the problem mathematically using the steady state of Equation (4).[15] After setting $P' = B' = 0$, one can solve $B = d_P P/d_B$ from Equation (4b), where P is the steady state of Equation (4a). If one were to know the ratio of the steady state number of peripheral cells over the number of bone marrow precursors, $R=B/P$, one can define the turnover in the bone marrow as $d_P = d_B(B/P) = d_B R$, which means that knowing the ratio R, the two labeling equations (5) and (6) would have only two free parameters, Δ and d_P that can reliably be estimated from the data.[15]

However, estimating the ratio, R, can be difficult because it remains unclear how many cells in the bone marrow can be counted as precursors. For neutrophils $R = 0.26$ has been defined as the ratio of the number of neutrophils in blood to the number of mitotic neutrophils in the bone marrow (which includes myeloblasts, promyelocytes, and myelocytes).[15] This is, however, based upon a model where the precursor cells are considered to be a single mitotic pool of self-renewing cells, from which cells randomly exit to a postmitotic pool.[15] Since the differentiation of neutrophil precursors in the bone marrow may in fact behave like a conveyer belt from dividing granulocyte-monocyte progenitors (GMPs) to myeloblasts, promyelocytes, and myelocytes, into the non-dividing postmitotic pool, it may be better to use a "chain model" where division is linked with differentiation, for example,

$$P_0' = s - d_0 P_0, P_1' = 2d_0 P_0 - d_1 P_1, P_2' = 2d_1 P_1 - d_2 P_2 \text{ and } B' = 2d_2 P_2(t-\Delta) - d_B B, \quad (7a\text{–}d)$$

where s defines the source from GMPs into the mitotic pool, and the various P_i subpopulations define an arbitrary number of stages within the mitotic pool. In this conveyer-belt model, the steady state of Equation (7d) is defined as $B = 2d_2 P_2/d_B$, which now only depends on the last precursor stage (here P_2), and not on the sum of all precursor cells. As a consequence, the ratio will be much smaller than the estimated R = 0.26, and we have limited knowledge on how to constrain R if we do not know how cell division and differentiation are linked during the development of neutrophil precursors in the bone marrow.

whether short-lived cells in the blood are produced by slowly cycling cells in the bone marrow, or whether longer lived cells in the blood are produced by rapidly cycling cells in the bone marrow (see Figure 2). Estimating the lifespan of cells in populations that are maintained by a source typically requires models that explicitly describe the labeling of the precursor cells in the source,[15-17] but also labeling data from that source. This issue is further addressed in Box 1 and we discuss it below.

Given the above-mentioned challenges linked to different labeling and modeling approaches and the remaining discrepancies in the literature, we here aim to provide a consensus about the average lifespans of different types of leukocytes in mice and men, and pinpoint which issues should be resolved to enable maximal use of this safe and non-invasive technology in the future.

2 | LABELING STUDIES CONSISTENTLY SHOW THAT NAIVE T CELLS LIVE MUCH LONGER THAN MEMORY T CELLS

In a series of papers, the turnover of naive and memory T cells in healthy individuals was estimated by short-term (10 hours-2 days) D_2-glucose labeling.[4,5,18,22-24] These studies consistently showed that, on average, naive $CD4^+$ and $CD8^+$ T cells live 5-10 times longer than memory $CD4^+$ and $CD8^+$ T cells.[5,22-24] Although these results may seem counterintuitive, they confirm early work by Michie et al,[25] who used the loss of unstable chromosomes in naive ($CD45RA^+$) and memory ($CD45RO^+$) human T cells after radiotherapy to study the turnover of naive and memory T cells. They observed that dicentric lesions remained present for more than a decade in the naive T-cell pool, and for less than a year in the memory T-cell pool. Although the study was based on markers that are now considered insufficient to sort naive T cells,[26] it was the first to demonstrate that in humans, immunological memory is maintained dynamically rather than by long-lived cells.[27]

We now know that some of these studies may have overestimated T-cell turnover rates because diurnal variation in deuterium availability was hardly taken into account.[12] Nevertheless, the relative naive and memory turnover rates within each study – which do not suffer from these problems – unequivocally show that memory T cells in the blood are shorter lived than their naive counterparts. This difference between naive and memory T-cell turnover was later confirmed in long-term deuterium-labeling studies.[10,18,28]

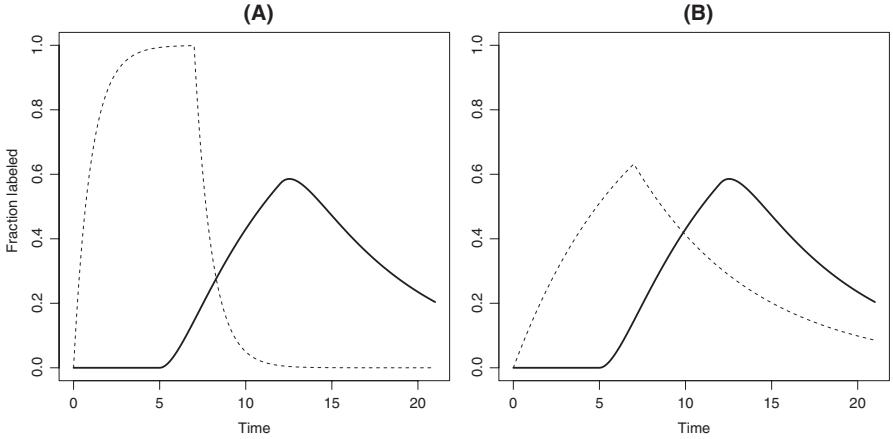

FIGURE 2 Labeling of the DNA of peripheral cells that are produced in the bone marrow. The dashed line depicts the fraction of labeled DNA in bone marrow precursors, l_P, as described by Equation (5), and the heavy solid line plots the fraction of labeled DNA in cells in the circulation, l_B, as described by Equation (6). Both equations were solved numerically for a labeling period of t_{end} = 7 days, and in both panels the time delay is Δ = 5 days. In Panel (A) the turnover of precursor cells in the bone marrow is fast, d_P = 1 per day, whereas that of their progeny in the circulation is slow, d_B = 1/7 per day (ie, an expected lifespan of 1 week). In Panel (B) the turnover of precursor cells in the bone marrow is slow, d_P = 1/7 per day, and that of their progeny in the circulation is fast, d_B = 1 per day (ie, an expected lifespan of 1 day). Despite the large difference in turnover of the circulating cells in panels (A) and (B), the two labeling curves in the periphery (ie, the heavy solid lines) are very similar to each other, because both curves follow the slowest time scale in the chain

3 | NAIVE T-CELL DYNAMICS IN HUMANS AND MICE

The first long-term deuterium-labeling study designed to estimate the average lifespan of naive $CD4^+$ and $CD8^+$ T cells in humans was published 15 years ago.[18] After 9 weeks of labeling, about 7% and 6% of the DNA of $CD4^+$ and $CD8^+$ naive ($CD45RA^+CD62L^+$) T cells was labeled. A simple exponential replacement model, $l(t) = 1 - e^{kt}$, where $l(t)$ is the observed fraction of labeled DNA and k is the replacement rate, suggested expected lifespans of 2.4 and 2.8 years for $CD4^+$ and $CD8^+$ naive T cells, respectively.[20] Because these volunteers were not followed after deuterium was withdrawn, we do not know how slowly these labeled naive T cells disappeared, which could have provided additional information on their expected lifespans.

One of our own long-term deuterium-labeling studies, which was published about a decade ago, reported that naive ($CD45RO^-CD27^+$) $CD4^+$ and $CD8^+$ T cells in young healthy human adults are expected to live for about 6 and 9.4 years, respectively.[10] In combination with very infrequent cell divisions, that is, about once every decade,[29] such long expected lifespans help to explain how naive T cells in healthy human adults are maintained for several decades even when thymic production has dramatically decreased.[30,31] The labeling data were originally fitted using the kinetic heterogeneity model with different turnover rates for unlabeled and labeled cells[14]; see Equation (1). Years later, when we started to use multi-exponential models (see Equation (2)), we found out that the data can also be described by a simpler kinetically homogeneous model[20,28,32] (ie, Equation (2) with $n = i = 1$). In fact, the even slower death rate of labeled cells reported by Vrisekoop et al[10] was a consequence of fitting the data with the too complicated model, and our conclusion that recently produced (ie, labeled) naive T cells are preferentially incorporated in the human naive T-cell pool was an overinterpretation. Importantly, the estimates for the expected lifespans of naive T cells were not affected by this.[20,28,32] In four of the healthy volunteers of the study by Vrisekoop et al,[10] deuterium enrichment in peripheral naive T cells was also determined 3-4 years after discontinuation of labeling.[32] The remaining deuterium enrichment level in these very long-term data points was perfectly in line with the predictions based on the data collected in the first year. Including these new data points from the healthy volunteers and fitting with an appropriate kinetically homogeneous model (ie, Equation (2) with n=i=1) revealed expected lifespans of $CD4^+$ and $CD8^+$ naive T cells of 5.5 (range: 3-9.1) and 9.1 (range: 4.6-27) years, respectively.[32] Although it remains unclear why the estimates made by Hellerstein et al[18] based on a very similar labeling approach were two- to threefold shorter than our own estimates, the presence of label in naive T cells 3-4 years after discontinuation of label administration [32] forms rather convincing evidence that individual naive T cells can survive for many years in healthy human adults.

3.1 | Naive T cells in aging humans

As thymic output is known to decline with age, several studies have addressed the question whether naive T cells have different dynamics in the elderly. Two studies reported very similar turnover rates of $CD45RA^+$ T cells in young and old individuals.[23,33] In our own D_2O labeling studies, we also found no statistically significant differences in the lifespans of $CD4^+$ naive ($CD45RO^-CD27^+$) T cells between healthy elderly and young adults.[28] The turnover rate of $CD8^+$ naive T cells, in contrast, was threefold faster in elderly volunteers compared to young adults.[28] This difference was probably caused by a

relatively high percentage of CD95$^+$ T cells in the CD8$^+$ T-cell pools of aged volunteers. Although we classified these cells as naive based on the expression of CD27 and the lack of expression of CD45RO, they may have included highly dynamic cells such as stem-cell memory (T_{SCM}) cells.[34] Indeed, based on their high Ki67 expression levels, these CD95$^+$ T cells turned out to divide much more frequently than their CD95$^-$ counterparts.[28] A more recent study, which performed 7 weeks of deuterium labeling and sorted naive CD4$^+$ and CD8$^+$ human T cells on the basis of CD27^{bright}CD45RO$^-$CCR7$^+$CD95$^-$, reconfirmed the very low levels of label incorporation of naive T cells and found no evidence for differences in the dynamics of naive T cells between young and old individuals.[34] Taken together, there is no evidence for a homeostatic response in the naive T-cell pool to compensate for reduced thymic output in the elderly.[28]

3.2 | Naive T-cell dynamics in mice

In mice, the population dynamics of naive T cells have also been studied with long-term deuterium labeling.[10,13,29,35] In a single experiment that was designed to study the effect of the length of the labeling period, naive (CD44$^-$CD62L$^+$) T cells were labeled for 1, 4 or 8 weeks.[13,35] Fitting the data with the kinetic heterogeneity model of Equation (1),[14] the resulting estimate for the average turnover rate of naive T cells hardly depended on the labeling period[13] and was about 0.023 per day for CD4$^+$ and 0.018 per day for CD8$^+$ naive T cells,[13] translating into expected lifespans of 43 and 56 days, respectively. The pool of naive T cells in mice is thus a lot more dynamic than in humans.[10,13,29,35] Another important difference between mouse and human naive T cells is that in mice the thymus remains largely responsible for the production of naive T cells throughout life, while in human adults, the majority of naive T cells are formed by peripheral division of naive T cells.[29]

As described above, deuterium-labeling data from human naive T cells tend to be described well by models for kinetically homogeneous cell populations, that is, assuming that all cells have the same turnover rate *d*. Interestingly, when labeling naive T cells in mice and fitting the data with the kinetic heterogeneity model of Equation (1),[14] we observed that the loss rate of labeled cells tended to decrease (albeit non-significantly, $P = 0.07$ for CD4$^+$ and $P = 0.19$ for CD8$^+$ naive T cells) when the labeling period increased,[13] suggesting that the naive T-cell compartment in mice may nevertheless contain some kinetic heterogeneity. It has previously been suggested that the naive T-cell pool may be composed of short-lived recent thymic emigrants (RTE) and longer lived mature naive (MN) T cells.[36,37] In an attempt to estimate the turnover rates of RTE and MN T cells, we uniformly labeled all cells in mice using a prenatal labeling protocol: deuterium labeling of female mice was started before conception, and after birth of their pups, deuterium was given to their offspring until 16 weeks of age, after which the loss of label from the different cell populations of the offspring was followed. In addition, we performed transplantation of congenic thymus lobes in order to track the fate of naive T cells egressing from the (transplanted) thymus.[35] These novel datasets were co-fitted with the 1, 4, and 8-week deuterium-labeling data using a mathematical model that allows part of the RTE to die before they mature into MN cells. We found no evidence for different death rates of RTE and MN T cells in the CD8$^+$ T-cell compartment, and fitting all data together using a kinetically homogeneous model (ie, Equation (1) with $n = i = 1$), we estimated that naive CD8$^+$ T cells have a turnover rate of .014 (95% confidence interval [CI]: 0.011-0.017) per day, translating into an expected lifespan of 70 (95% CI: 59-91) days. In contrast, in the CD4$^+$ naive T-cell compartment, the death rate of RTE (ie, 0.046 (95% CI: 0.028-0.081) per day) was threefold larger than the loss rate of MN T cells (ie, 0.015 (95% CI: 0.011-0.019) per day), and about half of the naive CD4$^+$ T cells were estimated to be RTE.[35] Since CD4$^+$ RTE are expected to gradually mature into MN T cells, thereby gradually decreasing their death rate, it would be better to model the naive CD4$^+$ T-cell population as a continuum and not as two discrete subpopulations. Nevertheless, these analyses suggest that CD4$^+$ RTE have a life expectancy of just 3 weeks (95% CI: 12-36 days), and that only an estimated 27% of them mature into naive T cells with a life expectancy of 2-3 months. The resulting overall life expectancy of CD4$^+$ naive T cells of $1/(0.5 \times 0.046 + 0.5 \times 0.015) = 33$ days is shorter than that of CD8$^+$ naive T cells, which is in good agreement with other mouse studies [13,29,38] as well as human studies.[10,28,32]

Hogan et al[38] suggested that the MN CD4$^+$ and CD8$^+$ T-cell pools in mice have another layer of kinetic heterogeneity, in that most cells are readily replaced by new RTE, while a subpopulation of long-lived "incumbent" cells are resistant to displacement by RTE and maintain themselves by peripheral division (with interdivision times of 167 and 213 days for CD4$^+$ and CD8$^+$ naive T cells, respectively, estimated with a model for Ki67 expression). Our data were also suggestive for the existence of such long-lived cells within the CD8$^+$ MN T-cell pool, as a relatively high enrichment of CD8$^+$ naive T cells was retained during the de-labeling phase of the prenatal labeling experiments. Since this long tail was absent from the CD4$^+$ prenatal labeling data, the existence of similarly long-lived CD4$^+$ naive T cells could not be confirmed.[35]

Summarizing, naive T cells have expected lifespans of 6-9 years in humans (see Table 1) and 2-3 months in mice. In human adults, naive T cells divide very infrequently to maintain the population at a relatively stable level, and to prevent a major loss of repertoire diversity despite a dramatic decline in thymic output.[28,39] In mice, naive T cells are a lot more dynamic and most naive T cells are produced by the thymus throughout life.[29]

4 | MEMORY T-CELL DYNAMICS IN HUMANS AND MICE

The life expectancy of circulating human CD45RO$^+$ memory T cells has been estimated in 9-week deuterium-labeling experiments.[10,13,18] Hellerstein et al[18] observed 17% enrichment in the CD4$^+$ and CD8$^+$ memory T-cell pools after 63 days of labeling. Using their precursor-product relationship, that is, $0.17 = 1 - e^{-k63}$, this translates into an expected lifespan of $1/k = 340$ days for both

TABLE 1 Lifespan estimates of different types of leukocytes[1]

Human	Subset	Markers	Estimate	Refs
T cells	Naive $CD4^+$	$CD45RO^-CD27^+$	5.5 yrs (range: 3-9.1)	[10,28,32]
		$CD45RA^+CD62L^+$	2.4 yrs	[18]
	Naive $CD8^+$	$CD45RO^-CD27^+$	9.1 yrs (range: 4.6-27)	[10,28,32]
		$CD45RA^+CD62L^+$	2.8 yrs	[18]
	Memory $CD4^+$	$CD45RO^+$	164 d (range: 71-500)	[13]
		All T cells except $CD45RA^+CD62L^+$	340 d	[18]
		$CD45RO^+CD25^-$	34 d (range based on SD: 21.6-81)	[33]
	Memory $CD8^+$	$CD45RO^+$	157 d (range: 113-231)	[13]
		All T cells except $CD45RA^+CD62L^+$	340 d	[18]
	T_{CM}	$CD8^+CD45RA^-CCR7^+CD28^+$	127 d[2] (range based on SD: 92-200)	[40]
	T_{EM}	$CD45RA^-CCR7^-CD28^{+/-}$	56 d[2]	[40]
		$CD45RA^-CCR7^-CD28^-$	84 d[2]	[40]
	T_{SCM}	$CD27^{bright}CD45RO^-CCR7^+CD95^+$	50 d (IQR: 27-62)	[34]
	T_{reg}	$CD4^+CD45RO^+CD25^{hi}$	12 d (range based on SD: 9.5-16.4)	[33]
	γδ		192 d (range: 83-714)	[28]
B cells	Total		52 d (range based on SD: 34-111)	[51]
			76 d (range based on SD: 47-208)	[52]
	Naive	$CD27^-$	217 d (range based on SD: 135-556)	[51]
		IgM^+CD27^-	435 (range: 278-625)	[28]
	Memory	$CD27^+$	38 d (range based on SD: 24-93)	[51]
		IgM^-CD27^+	44 d (range: 26-238)	[28]
	Natural effectors	IgM^+CD27^+	213 d (range: 143-283)	[28]
Neutrophils			19 hr^3 or 4.3 d	[15]
Monocytes	CM	$CD14^{++}CD16^-$	0.36 or 2.5 d	[16]
		$CD14^+ CD16^-$	1 d (SE: 0.26)	[17]
	IM	$CD14^{++}CD16^+$	0.9 d	[16]
		$CD14^+ CD16^+$	4.3 d (SE: 0.36)	[17]
	NCM	$CD14^+ CD16^{++}$	2.3 d	[16]
		$CD14^{lo}CD16^+$	7.4 d (SE: 0.53)	[17]

[1]The table does not cover all deuterium-labeling estimates from the literature. We have included neither D_2-glucose labeling studies that included the night (for reasons mentioned in the main text), nor naive T-cell estimates based on CD45RA only, nor D_2O labeling studies for neutrophils.
[2]Note that the average half-lives reported by Ladell et al[40] are slightly different, which is probably due to the fact that averaging turnover rates to compute an average half-life is not the same as averaging half-lives computed from turnover rates.
[3]The optimum favored by Lahoz-Beneytez et al.[15]

$CD4^+$ and $CD8^+$ memory T cells. The Vrisekoop et al data[10] were first fitted with the kinetic heterogeneity model (see Equation (1)).[14] Correcting for potential saturation effects by re-fitting these data with a multi-exponential model that explicitly describes kinetically different subpopulations (see Equation (2) and Figure 1), resulted in significantly better fits, and yielded average lifespans of 164 (range: 71-500) days, and 157 (range: 113-231) days for $CD4^+$ and $CD8^+$ memory T cells, respectively.[13] It is difficult to predict to what extent the estimates from Hellerstein et al[18] suffered from saturation effects, which could have led to an underestimation of memory T-cell turnover rates. We therefore conclude that on average, circulating memory T cells have a life-expectancy of months rather than weeks or years, again suggesting that long-lasting T-cell memory is due to relatively short-lived memory cells that maintain themselves by cell division.[25,27] Several studies have shown that the lifespan of memory T cells is remarkably similar between young and old individuals.[23,33]

4.1 | Memory T-cell subsets

In fact, the $CD45RO^+$ memory T-cell population consists of various phenotypically defined subpopulations, such as central memory

(T_{CM}) cells, effector memory (T_{EM}) cells, and effector (T_{EFF}) cells, which may all have different kinetics. Cells in the memory T-cell pool may also differ in the extent to which they are re-stimulated by their cognate antigens; some may be "true" memory T cells specific for pathogens that have been cleared a long time ago, while others may be chronically stimulated by latent viruses, food antigens, or bacterial antigens from the microbiome. The turnover of different subsets of memory T cells has been addressed in both short-term [22] and long-term [34,40] deuterium-labeling studies. When, in a 1-day D_2-glucose labeling study, cells were sorted into $CD4^+CD45RA^+CCR7^+$ naive (T_N), $CD4^+CD45RO^+CCR7^+$ T_{EM}, and $CD4^+CD45RO^+CCR7^-$ T_{CM} cells, it was shown that $CD4^+$ T_{EM} cells turn over threefold faster than their T_{CM} counterparts, and more than 20-fold faster than naive $CD4^+$ T cells,[22] which is in line with what was found in later long-term deuterium-labeling studies.

Ladell et al[40] performed 7 weeks of D_2O labeling in four healthy controls to study the average lifespans of $CD8^+$ T_{CM} and T_{EM} cells. The turnover rate of these cell types was computed from the exponential loss rate between two late samples from the de-labeling curve (week 10 and 18). Samples were taken late to guarantee that deuterium had been washed out from the body water. Assuming that these populations are kinetically homogeneous, this single exponential model translated into a loss rate of $CD45RA^-CCR7^+CD28^+$ T_{CM} cells in healthy controls of 0.0079 (with a standard deviation (SD) of 0.0029) per day, that is, an average lifespan of 127 (range based on the SD of the loss rate: 92-200) days. For $CD45RA^-CCR7^-CD28^{+/-}$ and $CD45RA^-CCR7^-CD28^-$ T_{EM} cells, Ladell et al[40] reported average turnover rates of 0.0177 (SD: 0.0217) and 0.0119 per day, translating into average lifespans of 56 and 84 days, respectively. These findings confirm the earlier finding that $CD8^+$ T_{EM} have shorter lifespans than T_{CM}[22] although standard deviations on the estimated parameters were large. Ladell et al[40] were able to recover labeled T_{EMRA} cells in only one of the healthy controls; the data obtained from that individual suggested that cells in this subpopulation are very long-lived.

It is important to note that the interpretation of the above labeling data of memory T-cell subsets is difficult because there is a whole differentiation pathway underlying the formation of memory cells. Cells may become labeled when dividing in one subpopulation of the memory pool and subsequently mature into a labeled member of a memory T-cell subset that is hardly dividing. When treating different memory T-cell subsets as independent subsets, one may therefore misinterpret the dynamics of the different cells. The exact differentiation pathway, which is often assumed to go from T_N via stem cell memory (T_{SCM}), T_{CM}, and T_{EM} to T_{EFF} cells,[41] is still debated. It may depend on the tissue location of the cells [42] and in fact mature phenotypes may even dedifferentiate into earlier phenotypes,[43] further complicating the interpretation of the data.

4.2 | Stem-cell memory T cells

Recently, Ahmed et al[34] performed a 7-week deuterium-labeling study, sorting $CD4^+$ and $CD8^+$ human T cells into $CD27^{bright}CD45RO^-CCR7^+CD95^-$ naive T cells, $CD27^{bright}CD45RO^-CCR7^+CD95^+$ T_{SCM} T cells, $CD45RA^+CD45RO^+$ transitional memory (T_{TM}) T cells, and $CD45RA^-$ memory $CD4^+$ and $CD8^+$ T cells. The data were fitted with various alternative models for the differentiation of these subsets (similar to Equations (4)-(6) in Box 1). T_{SCM} cells were found to accumulate more deuterium than T_{TM} cells and $CD45RA^-$ memory T cells. Based upon the modeling, it was concluded that T_{SCM} cells form a self-renewing population with rapid turnover, that is, with an average lifespan of 50 days (interquartile range (IQR): 27-62 days), which is at least threefold faster than the average lifespan of about 160 days in the human memory T-cell pool.[13] This rapid subset of T_{SCM} cells comprises 2%-3% of the circulating T-cell pool.[44]

4.3 | Regulatory T cells

Vukmanovic-Stejic et al[33] labeled four young adults and four elderly volunteers for 10 hours with D_2-glucose, to study the mechanisms whereby $CD4^+$ regulatory T cells (Treg) are maintained in healthy people. When $CD4^+$ T cells were sorted into $CD45RO^+CD25^{hi}$ "Treg" cells (which had very high levels of FOXP3 expression) and $CD45RO^+CD25^-$ memory T cells, they found in all volunteers that the Treg population became most strongly enriched in deuterium. They found average lifespans (based on the kinetic heterogeneity model (Equation (1)[14]) of 12 days (range based on SD: 9.5-16.4 days) for Treg cells and 34 days (range based on SD: 21.6-81 days) for memory T cells, suggesting that Treg cells turnover very rapidly, at a time scale of about 2 weeks. These estimated lifespans of memory T cells are unexpectedly short when compared to the other studies reviewed above. Since volunteers were labeled for 10 hours only, this study is not expected to suffer from underestimation of deuterium availability during the night,[12] and the reasons underlying this difference remain unresolved.

Based on the deuterium enrichment curves, Vukmanovic-Stejic et al[33] propose that Treg cells are highly dynamic and are maintained by extensive proliferation, either as Treg cells, or as memory cells differentiating into Treg cells. Indeed, they found that labeled (ie, recently formed) Treg cells are lost very rapidly. The short lifespan of Treg cells was further supported by their low expression of the antiapoptotic molecule Bcl-2, and their high susceptibility to apoptosis.[33] Because the repertoire of Treg cells largely overlapped with that of $CD4^+$ memory T cells, they proposed a model where a fraction of the memory T cells that are triggered to divide by persistent antigen develop into short-lived Treg cells, which die rapidly when their cognate antigen disappears.[33,45]

4.4 | Cognate memory T cells

There is some discussion in the literature as to whether T cells with a memory phenotype (such as the ones discussed above) are always true memory cells. For example, HIV-specific T cells with a memory phenotype are abundant in HIV-naive individuals,[46] and in naive specific-pathogen free (SPF) mice, T cells with a memory phenotype are formed very early in life.[29,35] Secondly, within the pool

of true memory T cells, there will be cells that chronically respond to persisting antigens and cells that are no longer exposed to their cognate antigen. Since all these memory T cells share the same phenotypic markers, the lifespan of each of these types of memory cells remained unknown. Recently, Akondy et al[47] reported on a long-term deuterium-labeling study to investigate the dynamics of cognate memory T cells in volunteers vaccinated against yellow fever (YF). The investigators were able to determine the deuterium enrichment in a relatively small $CD8^+$ memory $tetramer^+$ T-cell pool specific for this virus. In the memory phase, that is, 4-9 months after vaccination, YF-specific $CD8^+$ memory T-cell numbers were declining slowly, at a rate of 0.0057 per day. The fraction of labeled DNA in the cells during the same memory phase was also declining slowly due to the dilution of label by cell division, at an estimated rate of 0.0015 per day. Combining this rate of cell division with the net loss rate of the YV-specific T-cell population revealed a death rate of YV-specific memory T cells of 0.0057 + 0.0015 = 0.0072 per day, which corresponds to an expected lifespan of 139 days.[47] This longevity lies well within the range of the above-mentioned expected lifespan (157, range: 113-231 days) of bulk $CD8^+$ memory T cells in steady state.[13]

A major new finding was that—despite the average lifespan of YV-specific memory T cells of about 4 months—a large population of labeled memory cells persisted for years after vaccination, and that these persisting cells had a quiescent phenotype with features of stem-cell-like and effector-like memory $CD8^+$ T cells.[47] The latter finding suggests that either $CD8^+$ memory T cells slowly increase their longevity by becoming more quiescent over time, or that the population of antigen-specific $CD8^+$ memory T cells is kinetically heterogeneous, with the most quiescent subset becoming more predominant over time because they survive longer.[47] Another major new finding was that YV-specific memory cells had a very long intermitotic interval of 1/0.0015=666 days, that is, longer than their life expectancy. The authors proposed that the much shorter intermitotic times of bulk memory-phenotype T cells that have previously been reported are probably due to continuous stimulation by antigens.[47] A 10-hour D_2-glucose labeling study in 3 CMV-seropositive individuals suggested that even CMV-specific $CD8^+$ T cells, which are thought to be continuously exposed to their cognate antigen, had a longer life expectancy and longer intermitotic times than bulk memory $CD8^+$ T cells, which led the authors to conclude that the CMV-specific memory T-cell pool is expanding over time due to the accumulation of long-lived cells, and not due to ongoing rapid T-cell proliferation.[48] Future studies into the dynamics of memory T cells specific for different antigens, varying from those that are continuously presented to those that have disappeared from the body, are needed to gain more insight into how (desirable and undesirable) long-term memory T-cell responses against pathogens and self antigens are maintained.

4.5 | Memory T cells in mice

In analogy to naive T cells, which are a lot more dynamic in mice than in humans, our D_2O labeling studies have shown that mouse memory T cells in mice are a lot more dynamic than memory T cells in humans, and also more dynamic than mouse naive T cells. Using a multi-exponential model (Equation (2)) to simultaneously fit deuterium-labeling data from mice that were labeled for 1, 4, or 8 weeks or prenatally yielded expected lifespans of 14.8 (95% confidence interval (CI): 11.4-15.4) days for $CD4^+$ and 20.1 (95% CI: 11.7-22.0) days for $CD8^+$ memory ($CD44^+$) T cells.[13] These estimates are remarkably similar to those obtained by Younes et al,[49] who compared the turnover of lymphocytic choriomeningitis virus (LCMV)-specific $CD4^+$ memory T cells to that of $CD4^+$ "memory-phenotype" cells of unknown specificity using BrdU labeling. Of the memory-phenotype cells, 35% had picked up BrdU after 3 days and 60% after 10 days of labeling, which translates into an expected lifespan of 2-3 weeks.[20] The LCMV-specific $CD4^+$ T cells in contrast, divided approximately once every 50 days, suggesting that memory-phenotype $CD4^+$ T cells in mice (in line with what was observed for $CD8^+$ T cells in humans [47]) divide more frequently than "true" memory T cells in the absence of their cognate antigen.

Interesting data on the maintenance of cognate memory T cells in mice were provided by an experiment in which carboxyfluorescein succinimidyl ester (CFSE)-labeled LCMV-specific $CD8^+$ memory T cells, obtained 60 days after an acute infection with LCMV, were transferred into LCMV-naive recipient mice.[50] These cognate $CD8^+$ memory T cells were maintained at stable levels, and by modeling the slow CFSE dilution it was shown that the self-renewal of the memory T cells was accurately described by a Poisson process whereby individual memory T cells were involved in a single cell-division with an average inter-mitotic interval of 50 days.[50] Remarkably, data on unsorted $CD8^+$ memory T cells from LCMV-immune mice, which were CFSE labeled and transferred to LCMV-naive recipient mice, suggested that this slow stochastic renewal process was independent of the antigen specificity, as these cells were diluting CFSE at approximately the same rate. However, it remains unknown which fraction of these unsorted $CD8^+$ memory T cells were specific for LCMV, and hence true memory T cells.

Summarizing, in both mice and men, the majority of the circulating memory T cells are relatively short-lived.[13,49,50] In mice, cognate memory T cells maintain themselves by single self-renewal divisions occurring approximately every 50 days. In humans, part of the circulating memory T cells are quiescent and long-lived,[47] but it remains to be investigated what part of the memory T-cell pool is quiescent, how long-lived these cells are, and how this depends on the nature of the antigen and the location of the memory cells. See Table 1 for an overview of the absolute lifespans of human T cells reviewed above.

5 | B-CELL TURNOVER

A handful of in vivo deuterium-labeling studies have been performed to investigate how long-term B-cell memory is maintained. The very first study reporting on B-cell turnover in humans using in vivo deuterium labeling was Macallan et al,[51] who studied the kinetics of total, naive ($CD19^+CD27^-$) and memory ($CD19^+CD27^+$) B cells

in young and elderly healthy individuals using a 24-hours intravenous D_2-glucose labeling protocol and using the kinetic heterogeneity model proposed by Asquith et al[14] (Equation (1)) to interpret the data. The labeling kinetics of the total B-cell pool were surprisingly variable between individuals and differed more between individuals of the same age group than between young and elderly individuals.[51] In young adults, the total B-cell pool had an average turnover rate of 0.0193 per day, translating into an average lifespan of 52 days (range based on the SD of the average turnover rate: 34-111 days). Estimates in older individuals were not significantly different, with an estimated average lifespan of 65 days (range 35-500 days). The turnover rate of naive B cells (0.0046 per day, yielding an average lifespan of 217 days (range 135-556 days)), was much longer than that of memory B cells (0.0266 per day, ie, an average lifespan of 38 days (range 24-93 days)).[51] This study thereby gave the important insight that—in analogy to T cells—the memory B-cell population in the blood is maintained dynamically, not by cellular longevity. Indeed, of the two subpopulations, the naive B cells are the ones that proliferate relatively infrequently. Once activated, they undergo rapid proliferation, class switching, and somatic hypermutation and the resulting memory B-cell population is maintained as a pool of proliferating cells.[51]

The turnover rate of total B cells based on this 24-hour D_2-glucose labeling study was similar to the estimate reported in a later study based on 10 hours of oral D_2-glucose labeling, which reported a turnover rate of 0.0131 per day, translating into an average lifespan of 76 days (range based on the SD of the turnover rate: 47-208 days).[52] Also our own long-term (9-weeks) D_2O labeling study, which interpreted the data by fitting a multi-exponential model (Equation (2)), yielded similar turnover rates.[28] We found an average lifespan of 435 days (range: 278-625) for naive (IgM^+CD27^-), 44 days (range: 26-238) for memory (IgM^-CD27^+), and 213 days (range: 143-283) for natural effector (IgM^+CD27^+) B cells in young individuals. The estimates in older individuals were not significantly different.[28]

Despite the rather unambiguous results of these studies, they do not exclude the possibility that long-lived B cells contribute to the maintenance of long-term memory. Indeed, all the above studies were based on B cells isolated from human blood, and it is thought that long-term B-cell memory is in large part due to the long-term maintenance of plasma cells in the bone marrow.[53,54] Based on BrdU-labeling experiments in mice [53] and rhesus macaques,[55] these bone marrow plasma cells are thought to maintain themselves by cellular longevity, not by continuous renewal.

6 | NEUTROPHILS

Relative to B cells and T cells, neutrophils are known to be very short-lived cells. Based on radioactive tracers and adoptive transfer of cells, it has been estimated that neutrophils have a residence time of 4-18 hours in the blood (as reviewed in [15,56]). These results may have been confounded by direct effects from high-energy radioactive tracers, and by altered cell death or migration, particularly because neutrophils are very susceptible to apoptosis and altered cell trafficking upon ex vivo manipulation.[56] Since deuterium labeling does not have any of these side effects, it seems to be the ideal way to study the neutrophil residence time in peripheral blood.

The residence time of neutrophils in the blood has indeed been investigated in several in vivo deuterium-labeling studies.[15,57] The interpretation of the data is not trivial, however, and has raised quite some discussion.[15,58-60] There is consensus that neutrophils are produced by cycling precursor cells in the bone marrow, which enter a postmitotic pool, from which they enter the circulation after a time delay of 5 to 6 days. Neutrophils are subsequently thought to egress from the blood to enter peripheral tissues, may die and may even return to the bone marrow. The discussion revolves around the question how long neutrophils reside in the blood.

Labeling volunteers for 9 weeks with D_2O, Pillay et al[57] observed that the fraction of labeled DNA of neutrophils taken from the blood declined on a time scale of a few days, that is, much more slowly than the 4-18 hours loss rate estimated previously.[15,56] Fitting the data with a one-compartment model resembling Equation (2), with $n = i = 1$, we reported that human neutrophils reside in the blood for 5-6 days (and in the postmitotic pool for almost 6 days). A major problem associated with interpreting labeling data from cells that are produced in another compartment, here the bone marrow, is that this involves at least two timescales: (a) the division rate of cells in the bone marrow, and (b) the loss rate of cells in the circulation.[15,58,60] Thus, the slow time scale observed by Pillay et al[57] could in fact reflect a slow division rate of neutrophil precursors in the bone marrow, in combination with rapid turnover of neutrophils in the circulation (see Figure 2 for an example).

As mentioned above and explained in Box 1, the interpretation of deuterium-labeling data from cells that are produced elsewhere, here the bone marrow, requires mathematical models with at least two compartments, each having their own turnover rate (see Equations (4)-(6)). Considering the bone marrow as the compartment where precursor cells are produced by cell division, at a rate d_P, and the blood as the compartment where they are lost, at a rate d_B, the labeling curve in the blood will be dominated by the slowest of these two rates.[20,58] Fitting such a two-compartment model to the data obtained from the blood [57] yields two solutions: one with a relatively fast turnover rate of neutrophils in the bone marrow and a slow rate in the periphery, and another one with a relatively slow turnover rate in the bone marrow and a rapid rate in the blood (see Figure 2). Using a one-compartment model, we missed the latter solution.[57]

Lahoz-Beneytez et al[15] collected novel data using the more rapid, and thereby more appropriate, compound D_2-glucose. Since D_2-glucose was given for a maximum of 10 hours during daytime,[15] deuterium availability could be estimated well. By fitting a two-compartment model similar to Equations (4)-(6) to the experimental data, they indeed found the two optima described above, which both fitted the deuterium data well. In an attempt to decide which of the two solutions is the correct one, they estimated the ratio between the number of circulating neutrophils (B) and the number of dividing neutrophil precursors in the bone marrow (P, see Equation

(4)). This ratio ($R = B/P$) was substituted into the model describing the deuterium enrichment of neutrophils in the blood, which revealed a relationship between the turnover rate of neutrophil precursors in the bone marrow, d_P, and the turnover rate of neutrophils in the blood, d_B, that is, $d_P = d_B R$.[15] Thus, if this ratio is larger than one, the turnover in the bone marrow should be faster than that in the blood, and *vice versa*. Reviewing data on the population densities of neutrophils in the circulation and mitotic neutrophils in the bone marrow, that is, myeloblasts, promyelocytes, and myelocytes, they suggested that $R = 0.26$ (ie, there are roughly fourfold more neutrophil precursors in the bone marrow than mature neutrophils in the blood) and concluded that the turnover of neutrophils in the blood is faster than that in the bone marrow. The corresponding circulatory lifespan of neutrophils of 19 hours (SD: 6 hours)[15] is close to the traditional interpretation that the resident time of neutrophils in the blood is less than a day.

Importantly, by substituting the total number of mitotic neutrophils in the bone marrow it was implicitly assumed that all cells in the mitotic precursor pool, regardless of whether they are myeloblasts, promyelocytes, or myelocytes, enter the postmitotic pool with the same chance. If instead, division and differentiation were linked, that is, by cell division myeloblasts differentiate into promyelocytes and myelocytes, which enter the postmitotic pool following a final cell division, the number of precursors, P, in the above-mentioned ratio would be defined by the number of myelocytes undergoing their final cell division (see Equation (7)). Thus, the ratio R could in fact be much larger than 0.26, making it impossible to decide which of the two solutions is correct (our manuscript in preparation).

Since it is important to know which of the two interpretations of the deuterium data is correct, we strongly advocate in vivo labeling studies of neutrophil precursors in human bone marrow. Although this is a true challenge in humans, collecting bone marrow data is required to resolve this controversy; only modeling the bone marrow precursor pool in the absence of experimental data from that pool unfortunately does not suffice to resolve this issue.

7 | MONOCYTES

Like neutrophils, monocytes form a population of cells that are turning over quite rapidly and have as such been used as a fast control population in deuterium-labeling experiments. After 1 week of D_2-glucose labeling, the deuterium enrichment in monocytes in the blood approached 70%, corresponding to an estimated residence time in blood of 2-4 days.[7] Monocytes are heterogeneous, however, and have been subdivided into classical $CD14^{++}CD16^{-}$ (CM), intermediate $CD14^{++}CD16^{+}$ (IM), and non-classical $CD14^{+}CD16^{++}$ monocytes (NCM). The residence times of monocytes in these three subpopulations have recently been investigated in two independent D_2-glucose labeling studies.[16,17]

Both studies found that deuterium first accumulated in CM, and subsequently appeared in IM and later in NCM,[16,17] which confirmed the established linear differentiation scheme.[61] Patel et al[17] beautifully re-confirmed this pathway more directly by performing monocyte depletion experiments in humans and adoptive transfer experiments in humanized mice. For the interpretation of the deuterium enrichment curves, both studies used models resembling Equations (4)-(6), where mitotic progenitor cells in the bone marrow enter a postmitotic pool, which after some delay produces circulating CM, which may die, leave the blood, or become IM, which in turn may die, leave the circulation, or mature into NCM. Tak et al[16] considered models where the maturation from IM to NCM occurs inside or outside the blood (and found evidence for the latter). Patel et al[17] found no evidence for such maturation outside the circulation. According to both studies, NCM have the longest residence time in the blood, which agrees with the view that NCM form a population of blood-resident cells.[17]

Since monocyte samples were taken from the blood, while these cells picked up deuterium when they were produced by proliferation in the bone marrow, the interpretation of these two datasets suffers from the same uncertainty about the turn over of precursor cells in the bone marrow as the interpretation of the neutrophil data discussed above (see Figure 2). This problem was acknowledged by Tak et al,[16] who explicitly provided both solutions, that is, a CM residence time of 0.4 days (if blood monocytes turn over more rapidly than their bone marrow precursors) or 2.5 days (if blood monocytes turn over less rapidly than their bone marrow precursors). Patel et al[17] reported an average blood residence time of CM of about 1 day, but did not consistently choose for the first or the second optimum in all individuals. To reliably quantify the residence time of CM in the blood, one would need in vivo labeling data of the monocyte precursors in the bone marrow for the same reasons as described above for neutrophils.

Assuming the linear differentiation pathway is correct, fitting the data of IM and NCM reduces the problem of having two optima because the turnover rate of their "precursors" can be estimated from the labeling data of the less mature monocyte subsets in the blood. The residence times of IM and NCM can therefore be estimated with more certainty. Tak et al[16] estimated that IM have a residence time in the blood of about 0.9 days, after which they leave the blood and stay outside the circulation for about 1.6 days (standard error (SE): 0.26 days, with negligible cell death), before reentering the blood as NCM. Patel et al[17] reported a longer residence time in blood of about 4.3 days for IM. These estimates cannot be compared directly, because the underlying models make different assumptions about the survival of IM outside the circulation, that is, it seems natural that the model without reentry into the blood yields a longer residence time in the blood. For NCM, Tak et al[16] estimated a residence time of about 2.3 days, while Patel et al[17] reported 7.4 days (SE: 0.53 days). Although the reason for this threefold difference is again not totally clear, the time delay involved in the maturation from IM to NCM as modelled by[16] allowed for a better description of the data (compare Figure S2 of Patel et al[17] with Figure S3C of Tak et al[16]), which yielded a more rapid turnover rate of NCM.

Interestingly, recent single-cell RNA-sequencing data have suggested that IM do not form a homogeneous cell population, and that

monocytes tend to segregate into four clusters composed of classical and non-classical monocytes, a third novel cluster, and a fourth group expressing a cytotoxic gene signature (resembling "natural killer dendritic cells").[62] Since the IM subpopulation is overlapping with three of these clusters, it remains unclear how one should interpret the reported residence times of IM, and hence NCM, in the blood. This calls for repeating these deuterium-labeling studies by sorting the monocytes into the four clusters defined by Villani et al[62] and resolving the developmental pathway of these four cell types by studying the accumulation of deuterium in these subsets.

8 | DISCUSSION

This review summarizes the current best estimates for the life expectancies of various types of human and mouse leukocytes. Before we reported that estimates based on deuterium labeling vary remarkably between studies.[8] Since then, two important insights were obtained: (a) for kinetically heterogeneous cell populations it is important that the mathematical model that is used to fit the data explicitly describes the kinetically different subpopulations, and (b) normalization for the availability of label in the plasma is difficult in short-term D_2-glucose labeling studies that include the night. As both factors can have profound effects on the resulting estimated turnover rates of cells,[12,13] and since we have reviewed these studies before,[8] we here focused on studies of short-term D_2-glucose labeling that did not include the night or long-term D_2O labeling.

Each of the two methods (D_2-glucose and D_2O labeling) have their own merits. D_2O is the method of choice for long-lived cell types, such as naive T cells, because it can be administered over prolonged periods of time, allowing for reliable enrichment levels and for a time averaging over the various compartments that long-lived cells may be circulating through. Conversely, D_2-glucose is the method of choice for populations of short-lived cells such as neutrophils, which quickly approach high enrichment levels. A promising approach that should be considered in future D_2-glucose studies is to correct for the variability in plasma deuterium enrichment by closely scheduling and measuring food intake, frequently measuring plasma enrichment, and using a recently published physiologically based pharmacokinetic (PBPK) model to describe the changes in deuterium availability.[63]

Nevertheless, we are not there yet. Despite our quite stringent selection of studies, the summary in Table 1 reveals that cellular lifespan estimates still differ markedly. Although most studies agree that naive T cells live much longer than memory T cells, the estimated life expectancies of naive T cells differ from 2.4 to 9.1 years. It is perhaps not surprising that a 9-week labeling protocol cannot distinguish between these very long lifespans, but the finding that naive T cells still contained measurable levels of deuterium 3 to 4 years after stop of labeling convincingly shows that naive T cells in humans are very long-lived.[32] Actually, the very low rates of accrual and loss of deuterium in the naive T-cell populations described in Table 1 are quite surprising in the light of the recently described subpopulation of stem cell memory T cells (T_{SCM}), which share the same naive T-cell markers and were found to be very short-lived.[34]

Another interesting issue regarding the dynamics of naive T cells that needs to be investigated further is the difference in dynamics of naive T cells between SPF mice and men.[29] First, it remains unclear whether the dominant role of the thymus in the maintenance of the naive T-cell pool in laboratory mice is an artifact of the clean environment of these mice[64] or reflects a real difference between humans and mice. Additionally, it has been shown that in SPF mice there is a continuous large flux of cells from the naive T-cell compartment into the relatively small memory T-cell compartment,[65] which could be due to continuous priming of naive T cells by food antigens and/or the microbiome. In humans, we know little about the flux from the naive into the memory T-cell compartment, but if there were a significant contribution of the naive T-cell pool into the early phenotypes of the memory T-cell pool, we would need to account for that in mathematical models used for fitting labeling data of circulating memory T cells.

The turnover of memory T cells also remains quite ambiguous. Estimates between studies vary considerably (see Table 1) between 34 days and 340 days. While memory T-cell turnover may have been underestimated when using single-exponential models, it remains unclear why Vukmanovic et al[33] found a short lifespan of 34 days for $CD45RO^+CD25^-$ T cells, while Westera et al[13] found an average lifespan of 340 days for $CD45RO^+$ T cells. The kinetic heterogeneity of the memory T-cell population and uncertainties about their differentiation pathway complicate the estimation of their lifespans. For example, the fact that 5% of the cells expressing memory T-cell markers are actually Treg cells with an average lifespan of just 12 days,[33] will have increased average turnover rates of the total memory T-cell population and may thereby have masked the slower dynamics of other memory T cells. This calls not only for defining finer subsets in future labeling studies, but also for more explicit models for memory T-cell differentiation. Vukmanovic-Stejic et al,[33] for example, described a biology in which Treg cells mature from dividing memory T cells but fitted their data with a standard kinetic heterogeneity model.

Indeed, the seminal study by Akondy et al[47] suggested that some (antigen-experienced) memory T cells may be much longer lived than the average cell with a memory-phenotype. It would be very interesting to know whether true memory cells specific for other antigens show similar dynamics. The fact that the $CD8^+$ T-cell memory populations evoked by the yellow fever vaccine were not stable could depend on the antigen. The duration of B-cell memory after vaccination, for example, has been shown to depend on the nature of the vaccine,[66] and generally the tissue location of memory T cells will depend on the antigen and its route of vaccination or infection.[42,67,68] In SPF laboratory mice $CD8^+$ T-cell memory was stable after transfer of LCMV-specific memory T cells to naive recipient mice.[50] This stability could be due to a lack of novel infections in these mice, as existing memory may decline due to the establishment of novel memory cells.[69-72] Finally, many memory cells do not recirculate but reside in peripheral tissues and the bone marrow.[42] Hence, they tend to go unnoticed in samples taken from the blood.

Many memory T cells have been shown to reside as tissue-resident memory (T_{RM}) cells in the tissues originally affected by the pathogen, and it has been shown that memory T cells specific for pathogens causing systemic infections are overrepresented in the bone marrow.[67,68] It thus remains unclear how the average lifespan of half a year of human memory T cells in the blood should be interpreted, and deuterium-labeling studies of T_{RM} cells would be very valuable.

Disturbingly, our review indicates that short-term D_2-glucose labeling studies still tend to estimate faster turnover rates than long-term D_2O labeling studies (see Table 1, where short-term overnight D_2-glucose studies are excluded). In line with this, Lahoz-Beneytez et al[63] recently showed that taking the best estimate of deuterium availability in the plasma does not resolve all discrepancies in the literature. The reasons underlying these differences remain unclear and urgently need to be clarified.

A recurrent problem encountered in this review is the modeling of labeling data taken from circulating populations that are maintained by a source of cells from another compartment. Examples are neutrophils and monocytes that are produced in the bone marrow, naive T and B cells (partially) produced in the thymus and bone marrow, and cell types maturing into novel phenotypes that each may have their own turnover rates. Several studies have used "chain models" like Equation (7) to account for this [12,15-17,34] and have shown that a reliable interpretation of such data requires (labeling) information of the source compartment. Otherwise, these models have two equivalent solutions with either a slow or a fast source (see Figure 2). Deuterium-labeling data on the subpopulations within a differentiation pathway will provide essential biological information about the pathway when the enrichment increases sequentially in the various subsets, as was observed for the differentiation of monocytes.[16,17] This again calls for defining finer subsets in future labeling studies, and sampling precursor populations whenever possible. Additionally, when fitting deuterium-labeling data with kinetic heterogeneity models fails, for example, when the up-slope is steeper than the down-slope, this provides an indication that the cells are (partly) produced by a source from another population [our manuscript in preparation].

Modeling pathways becomes even more challenging when we realize that cellular differentiation may be linked with cell division. Consider, for example, a situation where entry of precursor cells into the postmitotic pool of the bone marrow is accompanied by a final cell division. This will lead to a much higher labeling peak in the blood a few days later (when these cells egress) than when their entry into the postmitotic pool occurs randomly (as most current models assume). The same is true for the maturation of circulating cell types, like memory T-cell subpopulations, where the rate of differentiation into the next phenotype may also depend on cell division. Future studies are needed that simultaneously sample from several subpopulations in differentiation chains, and use mathematical models based upon alternative biological assumptions to explain the data. This emphasizes that this field needs proficient collaborations between modelers and immunologists, as both groups need to appreciate the underlying biological uncertainties and the importance of choosing an appropriate mathematical model.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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