Lymphocyte kinetics: the interpretation of labelling data

Becca Asquith, Christophe Debacq, Derek C. Macallan, Luc Willems and Charles R. M. Bangham

DNA labelling provides an exciting tool for elucidating the *in vivo* dynamics of lymphocytes. However, the kinetics of label incorporation and loss are complex and results can depend on the method of interpretation. Here we describe two approaches to interpreting labelling data. Both seek to explain the common observation that the estimated death rate of lymphocytes is higher than their estimated proliferation rate. In the first approach, an additional source of lymphocytes is postulated. In the second, it is maintained that lymphocyte heterogeneity is sufficient to account for the observation. We explain why we favour the second approach, arguing that the addition of a large source of lymphocytes is unnecessary and difficult to reconcile with what is currently known about lymphocyte physiology. We discuss how the choice of model can affect data interpretation.

Becca Asquith
Charles R. M. Bangham*
Dept of Immunology, Faculty of Medicine, Imperial College, Norfolk Place, London, UK W2 1PG.
*e-mail: c.bangham@ic.ac.uk

Christophe Debacq
Luc Willems
Molecular and Cellular Biology, Faculté Universitaire des Sciences Agronomiques (FUSAGx), 13 avenue Maréchal Juin, 5030 Gembloux, Belgium.

Derek C. Macallan
Dept of Infectious Diseases, St George's Hospital Medical School, Cranmer Terrace, London, UK SW17 0RE.

If lymphocyte kinetics – the proportion of lymphocytes that proliferate and that die in a day – could be estimated *in vivo*, then this would be a vital step in the understanding of many diverse aspects of immunology. Maintenance of immune memory, T-cell homeostasis, regulation of the adaptive immune response during viral infection and the pathogenesis of $CD4^+$ depletion in HIV infection: an understanding of all of these would be a step closer. DNA labelling *in vivo* has the potential to elucidate lymphocyte kinetics in this way. However, the dynamics of label incorporation and loss are complex [1] and it is easy to misinterpret the data. We will discuss one aspect of DNA label interpretation that has caused much debate: the assumption of kinetic homogeneity in lymphocyte populations [2–4]. We compare two different approaches to the problem, explaining why we favour one approach and expanding on the consequences of this interpretation.

DNA labelling techniques: BrdU and ^{2}H glucose

The labelling of DNA with bromodeoxyuridine (BrdU) is a common technique [5], which is widely used in animals but is generally considered unsuitable for use in humans because of its toxicity. The recent development [6] of a stable, non-toxic label suitable for use in humans, deuterated glucose (^{2}H glucose), has opened the door to a true *in vivo* understanding of human lymphocyte kinetics.

BrdU and ^{2}H glucose are both incorporated into the DNA of dividing cells. BrdU is a nucleoside analogue that is incorporated in the place of thymidine when a cell divides during the labelling period. Cells that contain BrdU can be detected and enumerated using flow cytometry [5]. By contrast, ^{2}H glucose labels the pentose sugar (rather than the base) of nucleosides synthesized during the labelling period. The ratio of labelled to unlabelled nucleosides can be measured using gas-chromatography mass-spectrometry [6].

In both BrdU and ^{2}H glucose techniques, the amount of label is measured over time following a labelling period. The data generated, showing acquisition and loss of label, is used to infer the kinetics of the cell population studied (Box 1). Crucially, DNA labelling techniques only label cells that have divided during the labelling period (and in the case of BrdU, their progeny). This is in contrast to non-DNA labelling techniques, such as chromosome damage or carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling, which label cells regardless of division history.

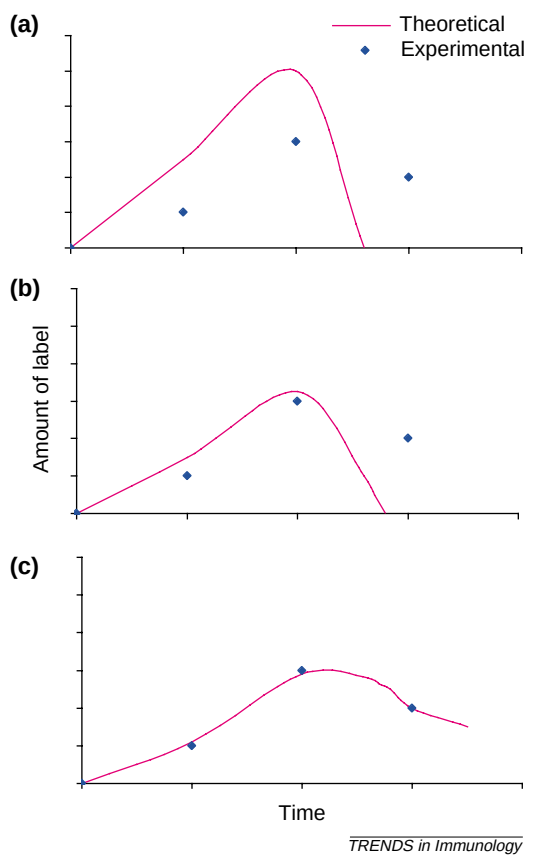
Cell populations of constant size

In healthy adults, the size of many lymphocyte populations (e.g. B cells, $CD8^+$ T cells, $CD4^+$ $CD45RO^+$ T cells) is approximately constant, or changes only very slowly with time. Similarly, in several chronic viral infections, for example, HIV-1 and HTLV-1 (human T-cell lymphotropic virus-1), the number of infected lymphocytes and the magnitude of the cellular immune response to the virus are approximately constant. If a lymphocyte population is of constant size, then the flow of cells into that population must be balanced by the flow of cells out of that population. Input of cells into a lymphocyte population includes proliferation in the periphery (antigen-driven and homeostatic proliferation), production in the thymus and bone marrow and maturation from different cell populations. Loss of cells from a lymphocyte population results from cell death (including cell senescence, activation-induced cell death, death induced by cytopathic viruses) and cell maturation.

In the case of lymphocytes in adults, peripheral proliferation and cell death are thought to account for the largest changes in cell numbers, with maturation and thymic export playing smaller roles. It would therefore be expected that, for a population of constant size, the proliferation rate and the death rate of lymphocytes measured in labelling studies would be approximately equal. This does not appear to be the case. Instead, it is a common feature of BrdU [7–9] and ^{2}H glucose [10] labelling studies that the estimated death rate is considerably higher than the estimated proliferation rate. Two different explanations for this discrepancy have been postulated: the existence of a source term and the existence of kinetically heterogeneous

 1471-4906/02/$ – see front matter PII: S1471-4906(02)02337-2

Box 1. Inferring proliferation rates and death rates from labelling data



Step 1: Construct a mathematical model to describe the labelling experiment.
A model is devised that predicts a labelling time course for a given value of the proliferation rate and the death rate.
Step 2: Fit the model to the data.
The aim of this step is to find the values of the proliferation rate and the death rate that make the theoretical prediction of the model come closest to the observed experimental data (Fig. I).
This is done by varying the values of the proliferation rate and death rate in the model to see which combination of parameters gives the best fit.
Step 3: Produce best estimates of the proliferation and death rates
The best estimates of the proliferation and death rates are those that yield the best fit of the model to the data.
In this case the best estimates would be $p = 0.05$ and $d = 0.1$.
Step 4: Check the effects of changing the model assumptions.
Any mathematical model will be too simple to describe a biological system – it will contain a number of assumptions. It is important to check that relaxing these assumptions does not significantly alter the estimate of p and d.

Fig. I. Initially, the proliferation rate and death rates are guessed ($p = 0.1$, $d = 0.2$). These values are substituted into the model. This produces a theoretical labelling curve. The theoretical labelling curve is compared with the experimental data. In this example (a), it can be seen that the theoretical curve is too high. The proliferation rate is therefore decreased ($p = 0.05$, $d = 0.2$) and these values are substituted into the model. This produces another, lower, theoretical labelling curve (b). Although the fit is improved, it can be seen that the theoretical loss of label is too rapid. The death rate is therefore decreased ($p = 0.05$, $d = 0.1$) and these values are substituted into the model. This produces another theoretical labelling curve (c). These estimates of the proliferation rate and death rate ($p = 0.05$, $d = 0.1$) give the best fit of the model to the data. In practice this step would be performed by a computer with several hundred combinations of p and d considered.

subpopulations. These two models are summarized in Fig. 1.

Source term

In this approach [7,10], it is argued that because the death rate of the population is higher than the proliferation rate, an additional input of cells must be postulated in order to maintain the constant size of the population. This additional input of cells is referred to as the 'source' and 'represents replenishment of T cells by the thymus, extrathymic sites or a subpopulation of peripheral T cells that can be recruited into active division' [7]. However, the measurements made on the source do not appear compatible with this description. The production of T cells by the source is extremely large: in human controls it is between 3 times and 30 times higher than the contribution from peripheral proliferation [10]. If the source represented the thymus then the conclusion would be that the thymus released some 10^{10} T cells per day. The exact level of thymic export in adults is unknown but current estimates are of the order of 10^8 T cells per day [11,12]: considerably lower than the output of the source described by Mohri *et al.* [10]. Furthermore, a discrepancy between the death rate and the proliferation rate is observed in $CD45RO^+$ as well as $CD45RA^+$ cells (Macallan *et al.*, unpublished). This cannot be attributed to thymic export, which would mainly produce $CD45RA^+$ cells. Similarly, extrathymic sites are unlikely to contribute so many lymphocytes per day, because their progeny are mainly restricted to non-classical T cells [11], which are a very small proportion of circulating lymphocytes. The possibility remains that the source represents the replenishment of activated cells by a non-dividing or slowly dividing subpopulation that have undergone clonal expansion on activation [8]. However, if the subpopulation of cells were non-dividing then they could not provide a constant net output of cells: that is, they could not provide a source (this can be seen explicitly in Bonhoeffer *et al.* [8], Eqn 12 and 13, when p' is set equal to zero). If the subpopulation of cells were slowly dividing then they could provide a net output or source but it would be expected that all of the cells in this source would incorporate label during the labelling period. This is not observed [10]. Even if the source were taken to be the sum of thymic output, extrathymic output and peripheral proliferation there is still a discrepancy between this description and the output of cells attributed to it [10]. For the reason described peripheral proliferation can only contribute labelled cells;

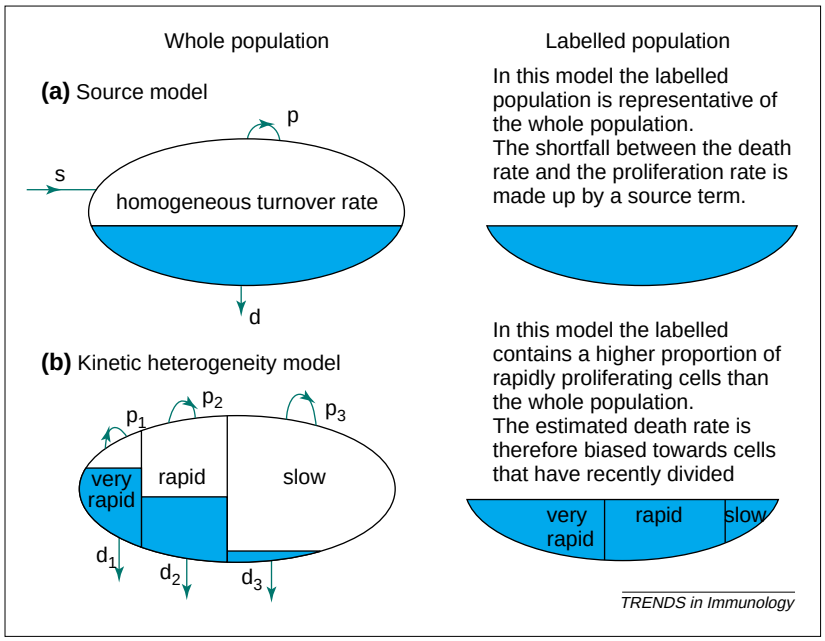


Fig. 1. Source model and kinetic heterogeneity model compared. The whole population of interest is represented on the left, the fraction of this population that becomes labelled is represented on the right. Blue shading denotes label. (a) In the source model because the population is assumed to be homogeneous, the labelled population is assumed to be a representative sample of the whole population. It therefore has the same kinetics (proliferation rate and death rate) as the whole population. (b) In the kinetic heterogeneity model, the population is assumed to be heterogeneous (in the illustration there are three subpopulations with different kinetics). The labelled population is therefore, not a representative sample of the whole population. Instead it is biased towards the rapidly proliferating cells and its kinetics, therefore, differ from those of the whole population.

unlabelled cells must be contributed by the thymus and extra thymic sites. Yet in the control subjects studied by Mohri *et al.* [10], an average of 84% of $CD4^+$ cells and 99.6% of $CD8^+$ cells in the source are unlabelled. That is, with a 2:1 CD4:CD8 ratio, the source produces approximately $(^2/_3 \times 0.84 + {}^1/_3 \times 0.996) \times 10^{10} = 0.89 \times 10^{10}$ unlabelled cells. This is still far in excess of current estimates of thymic and extrathymic output (moreover, it would be expected that a proportion of cells exported from the thymus would have divided – further decreasing the estimate of unlabelled cells that could be produced by the thymus). It is therefore difficult to identify the physiological correlate of such a large, partially labelled source.

Kinetically heterogeneous subpopulations

In the second approach [9], it is argued that the disparity between the estimated proliferation rate and the estimated death rate is entirely consistent with a population of constant size, provided that the population is not completely homogeneous. Here, we define a kinetically heterogeneous population to be one that consists of cells or subpopulations of cells with different kinetics.

Because the population examined is not kinetically homogeneous, the labelled fraction is not a representative sample of the whole population. Instead the labelled fraction only contains cells that divided recently; it is therefore biased towards rapidly proliferating cells. Because DNA labelling techniques measure the proliferation rate of one population (the whole population) and the death rate of another (the labelled population) it is not surprising that there is a discrepancy between the two rates, despite the fact that the population as a whole is of constant size. In general, it would be expected that the measured death rate would be greater than the measured proliferation rate because the death rate measured is that of cells that turn over (proliferate and die) most rapidly. This is in agreement with numerous studies using DNA-labelling techniques [7,9,10]. An example to illustrate this is given in Box 2. In the case of cell populations where recent proliferation is not correlated with a higher probability of death, for example, cells that have a

Box 2. Numerical example to illustrate the effect of kinetic heterogeneity in lymphocytes

Imagine an experiment to measure the kinetics of a $CD4^+$ lymphocyte population. The $CD4^+$ population will contain several subpopulations with different kinetics (e.g. $CD45RA^+$ cells, $CD45RO^+$ cells, $CCR7^+$ cells, resting cells, activated cells, cells that have recently met cognate antigen, cells that have recently met stimulating cytokines).

For the sake of simplicity, imagine that the $CD4^+$ population is made up of just two subpopulations: subpopulation X and subpopulation Y. Let subpopulation X be a slowly turning over subpopulation making up 90% of the $CD4^+$ cells; let its proliferation rate be 0.02 day^{-1} and its death rate be 0.02 day^{-1} (i.e. 2% of cells in population X proliferate in a day and 2% die in a day). Let subpopulation Y be a rapidly turning over subpopulation making up the remaining 10% of the $CD4^+$ cells; let its proliferation rate be 0.2 day^{-1} and its death rate be 0.2 day^{-1} (i.e. 20% of cells in population Y proliferate in a day and 20% die in a day). The average proliferation rate of the whole $CD4^+$ population is therefore, $90\% \times 0.02 + 10\% \times 0.2 = 0.038$ day^{-1}. Similarly, the average death rate of the whole $CD4^+$ population is $90\% \times 0.02 + 10\% \times 0.2 = 0.038$ day^{-1}. Note that in this example, the $CD4^+$ population is in equilibrium, that is, of constant size because the average death rate equals the average proliferation rate.

Imagine that this $CD4^+$ population is labelled for a finite time with a DNA label that is only taken up when a cell divides. The proportion of cells from the rapidly turning over subpopulation Y will be higher in the labelled population than in the whole population because more of the rapidly dividing cells will have divided during the labelling period. In the labelled pool, if 60% are from subpopulation X and 40% are from subpopulation Y, the average death rate of the labelled pool will be $60\% \times 0.02 + 40\% \times 0.2 = 0.09 day^{-1}$. Similarly the average proliferation rate of the labelled pool is $60\% \times 0.02 + 40\% \times 0.2 = 0.09$ day^{-1}.

DNA labelling experiments measure the average proliferation rate of the whole population (0.038 day^{-1}) and the average death rate of the labelled population (0.09 day^{-1}).

It can clearly be seen that despite the population being in equilibrium the measured death rate is far in excess of the measured proliferation rate.

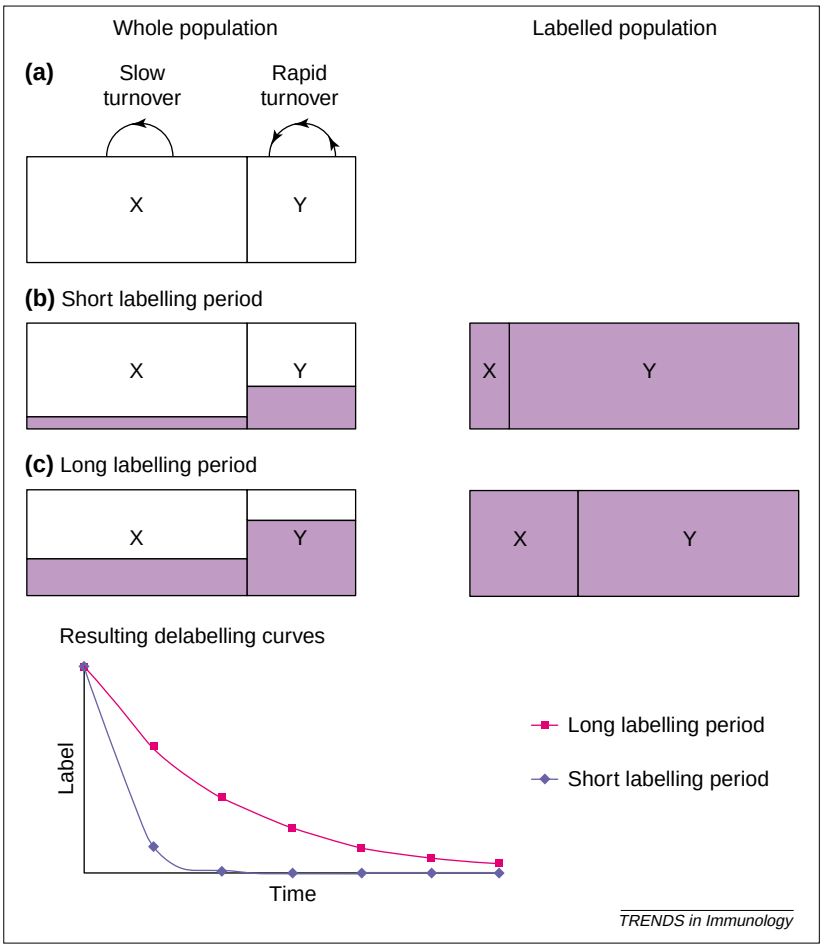


Fig. 2. Measured death rate varies with the length of the labelling period. Death rates are estimated from the kinetics of labelled cells. The death rate measured is therefore, the death rate of labelled cells. As the length of the labelling period is varied, the subpopulation structure of the labelled cells varies and therefore, the estimated death rate varies also. Consider a population that consists of just two clonal subpopulations X and Y. (a) Assume that both populations are of constant size and that the larger population, population X, has slower dynamics (i.e. the proliferation and death rate of population X is less than that of population Y). After a short labelling period (b) a higher proportion of cells from Y are labelled (purple shading) because Y proliferates more rapidly than X. Therefore, the ratio of population X to Y in the labelled cells (right hand column) does not reflect the ratio of population X to Y in the whole population (left hand column). The labelled cells, therefore, exhibit more rapid delabelling kinetics than they would if the whole population was labelled. Consequently, the measured death rate is higher than the average death rate. If the length of the labelling period is increased (c) the constituents of the labelled population more closely represent that of the whole population and the death rate measured is closer to the average death rate.

fixed lifespan, the measured death rate would not be expected to be greater than the measured proliferation rate.

This explanation is consistent with what is known about normal lymphocyte physiology. T-cell and B-cell populations are clonally diverse: it is therefore probable that they contain subpopulations with different kinetics depending on level of receptor expression, phenotype, cytokine microenvironment, and whether or not they have recently met cognate antigen.

It is currently not possible to be certain which of these two approaches (discussed earlier and illustrated in Fig. 1) best explains the observed discrepancy between estimated proliferation and estimated death rates. However, we favour kinetic heterogeneity as the most probable explanation. Indeed, one author of a paper advocating the source theory has recently published a letter suggesting that lymphocyte heterogeneity might be a more appropriate interpretation [3]. Therefore, although we cannot rule it out, we do not consider the source model any further and instead focus on the kinetic heterogeneity theory.

Consequences of kinetic heterogeneity

One important consequence of the kinetic heterogeneity model is that the average death rate of a population cannot be directly measured with DNA labelling techniques unless the entire population incorporates label. Instead, the death rate estimated is that of labelled cells only. The death rate measured is, therefore, a function of the labelling protocol, in particular the length of the labelling period (Fig. 2).

Death rate estimates obtained using DNA-labelling techniques would be expected to decrease as the length of the labelling period increases. It is difficult to find truly comparable data but a very preliminary meta-analysis suggests that this might be the case (Fig. 3). Further investigations on more comparable populations are necessary to ascertain if this is a genuine result.

Better estimates of the average death rate could, in theory, be obtained using non-DNA labelling techniques (e.g. CFSE or chromosome damage [13,14]). This is because labelling techniques that do not rely on cell division for label uptake do not favour labelling of cells with rapid turnover rates. Consequently the labelled fraction is a more representative sample of the population as a whole. However, both radiation treatment and standard CFSE labelling protocols (involving the labeling of cells *ex vivo* followed by their reinfusion into the subject) are of limited use in humans and involve substantial manipulation of the cells making it difficult to know how useful non-DNA labelling techniques are in practice. Alternatively, the average death rate of the whole population could be indirectly inferred from DNA labelling data because, for a population of constant size, the average death rate is equal to the average proliferation rate (assuming that disappearance of labelled cells by mechanisms other than death is negligible).

Once it is appreciated that the death rate measured is not the average death rate of the whole population but instead the death rate of the labelled population, then this can be turned into an advantage. By systematically varying the length of the labelling period the subpopulations 'seen' by labelling experiments will change and information about the kinetics of different subpopulations can be obtained (Fig. 2). Interesting data can also be obtained by analysing label enrichment in cell populations sorted for markers of apoptosis (Macallan *et al.*, unpublished).

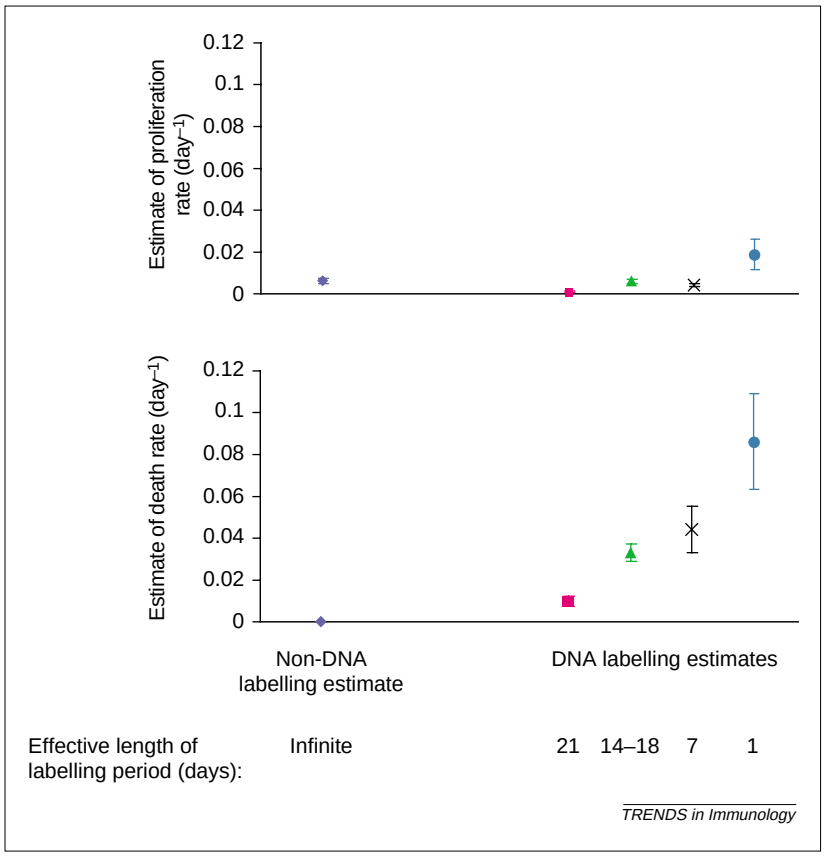


Fig. 3. Length of labelling period might be inversely correlated with death rate estimates. Estimates of the proliferation and death rate of $CD4^+$ T cells in normal subjects obtained in five different experiments are shown. Although there is broad agreement in the proliferation rate estimates, the death rate estimates vary more widely and appear to be inversely correlated with the length of the labelling period. It is difficult to make rigorous comparisons across different subject groups, experimental and mathematical methods and so this analysis should be treated extremely cautiously. However, it does provide a plausible explanation for at least some of the between experiment variation. Source of parameter estimates: navy diamond, [13]; red square, [7]; green triangle, [16]; black cross, [10]; blue circle, (Macallan *et al.*, unpublished).

Modelling kinetic heterogeneity

The obvious way to model kinetic heterogeneity would be to explicitly model each kinetic subpopulation separately. This approach would be most appropriate if the identity of the main subpopulations was known (and their individual kinetics were of interest). However, in general, explicitly modelling each subpopulation is problematic because it would involve the introduction of at least two free parameters† for each subpopulation considered (turnover rate of the subpopulation and size of the subpopulation). Furthermore, these parameters would be highly correlated and potentially limitless in number because division into subpopulations could probably be continued *ad infinitum* depending on the degree of resolution of the different subpopulations.

†A free parameter is an unknown variable (e.g. proliferation rate or death rate) that is to be estimated from the data. The more free parameters there are the harder it is to estimate any of them with confidence. This is particularly true if the parameters are highly correlated or if there are few data points.

Acknowledgements
This work was supported by the Wellcome Trust and Fortis Bank Assurance. B.A. is a Wellcome Trust Research Fellow (ref: 054451), C.D. is a fellow of the Action de Recherche Concertée du Ministère de la Communauté Française and L.W. is a research director of Fonds National de la Recherche Scientifique. We would like to thank Angelina Mosley for interesting and useful discussions and anonymous referees for some very constructive comments.

Instead, another method of modelling kinetic heterogeneity can be used [9]. In this approach there are just two free parameters to estimate: the average proliferation rate of the whole population and the death rate of labelled cells. An example of this approach, as applied to deuterated glucose labelling in a population of constant size, is given in Box 3.

Conclusions

When DNA labelling techniques are employed to investigate *in vivo* lymphocyte kinetics it is commonly observed that estimated death rates exceed estimated proliferation. Here, we have compared two possible interpretations of this observation. In one interpretation a 'source' term is included in the analysis to compensate for the proliferation versus death rate discrepancy. In the alternative interpretation it is assumed that the discrepancy can be explained by variations in kinetics of the subpopulations that constitute the population as a whole. Both interpretations are consistent with a population of constant size.

The choice of approach has profound effects on the results of DNA labelling experiments. Not only will the two methods yield different proliferation rates, more importantly, they could yield fundamentally different physiological interpretations of data. Proliferation rates will be different because in the source model label in the source itself does not contribute to the proliferation rate estimate (although it must have originated from cell proliferation); the source model will therefore, give lower proliferation rate estimates. More significantly, data interpretation can also differ according to the approach. Here, as an example, we discuss how the results of a recent experiment [10] are affected by the choice of mathematical model. Mohri *et al.* [10] studied seven HIV-1 infected subjects and four uninfected controls using the ^2H glucose technique. The resulting data were analyzed using the source model. In the infected subjects a correlation was observed between the estimated proliferation rate of $CD8^+$ cells and Ki67 expression (a cell-cycle antigen). Surprisingly, there was no such correlation between the estimated death rate and TUNEL positivity (an indicator of apoptosis) in $CD8^+$ cells. This cannot be explained in the context of the source model that Mohri *et al.* use unless it is supposed that most $CD8^+$ cell death is not by apoptosis. However, re-examined in the light of the kinetic heterogeneity model, the lack of correlation is not surprising because the death rate measured is not that of all $CD8^+$ cells but only of labelled cells, which are not a representative sample of the whole. However, the proliferation rate measured is the proliferation rate of the whole $CD8^+$ population and therefore, would be expected to be correlated with Ki67 expression on the whole population. If apoptosis was the main mechanism of $CD8^+$ cell death in

Box 3. Possible model for interpreting ^{2}H glucose data in a population of constant size

Consider the analysis of data from an experiment in which the ratio of labelled to total deoxyadenosine was measured over time in a population of constant size.

We model the rate of change of total deoxyadenosine (A) and labelled deoxyadenosine (A*) with time, in terms of two free parameters: p the average proliferation rate and d* the disappearance rate of labelled cells. Total deoxyadenosine is proportional to the number of cells in the population and is therefore constant with time. Labelled deoxyadenosine is increased when cells proliferate at a rate p and lost when labelled cells disappear (die, change phenotype, migrate) at a rate d*. The equations to describe this system are, therefore:

$$\frac{dA}{dt} = 0$$

$$\frac{dA^*}{dt} = bpA - A^* d^* \quad t \leq \tau \text{ during the labelling period}$$

$$\frac{dA^*}{dt} = -A^* d^* \quad t > \tau \text{ after the labelling period}$$

where b is the probability that an incorporated deoxyadenosine molecule will be labelled and τ is the length of the labelling period. Solving these equations analytically yields

$$\text{enrichment fraction} = \frac{A^*}{Ab} = \begin{cases} \frac{p}{d^*}(1 - e^{-d^* t}) & \text{during the labelling period} \\ \frac{p}{d^*}(1 - e^{-d^* \tau})e^{-d^*(t-\tau)} & \text{after the labelling period} \end{cases}$$

This model can then be fitted to experimental data (Macallan *et al.*, unpublished). An equivalent approach to modeling BrdU data has been used [a]. It should be noted that the kinetic heterogeneity model is not equivalent to the source model even if the source is slowly dividing cells (see discussion about the source model on why the source model is incompatible with data and see conclusion for examples of how interpretations of data vary between the two models).

Reference

a Debacq *et al.* (2002) Increased cell proliferation but not reduced cell death, induces lymphocytosis in bovine leukemia virus-infected sheep *Proc. Natl. Acad. Sci. U. S. A.* 99, 10048–10053

recently divided cells, then there would be a positive correlation between the estimated death rate and TUNEL positivity in labelled CD8$^+$ cells. It would be interesting to compare the correlation between estimated death rates and TUNEL positivity in labelled CD8$^+$ and CD4$^+$ cells: it could be determined if death in recently divided cells (some 10–20% of the population for a 7-day labelling period [10]) was mainly as a result of apoptosis (e.g. activation induced cell death or CTL lysis) or mainly as a result of non-apoptotic death (e.g. the cytopathic effects of HIV-1).

Another interesting result reported by Mohri *et al.* was the observation that HIV-1-infected subjects have a larger source term for CD4$^+$ cells than uninfected controls but a smaller source term for CD8$^+$ cells. In the context of the source model, an increase in the source implies that thymic output, extrathymic output or activation of resting cells has increased. In the context of the kinetic heterogeneity model, an increase in the source (i.e. a greater discrepancy between the death rate and the proliferation rate) implies that the population is more heterogeneous, that is, that there is a greater difference between the labelled population and the whole population. So, for instance, the observation that the CD8$^+$ cell source is decreased in HIV infection is interpreted in the context of the source model to mean that thymic output is decreased by infection or that fewer resting CD8$^+$ cells become activated during infection (a prediction that is hard to reconcile with the gradual decrease observed in resting CD8$^+$ cell numbers [15]). Conversely, in the context of the kinetic heterogeneity model, Mohri's result implies something very different: in infected patients the entire CD8$^+$ pool more closely resembles the rapidly turning over subpopulation in uninfected patients, suggesting that there is a high degree of activation across the whole CD8$^+$ cell population. It can therefore be seen that data interpretation is dependent on the choice of model.

Given the dramatic effect that the choice of model can have on DNA labelling interpretation, it is vital to examine carefully which model is more appropriate. Although the source model cannot be ruled out, we believe that, on the balance of current evidence, the kinetic heterogeneity model is more likely to provide an accurate description of DNA labelling.

References

1 Hellerstein, M.K. (1999) Measurement of T-cell kinetics: recent methodologic advances. *Immunol. Today* 20, 438–441
2 Grossman, Z. *et al.* (2002) CD4$^+$ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat. Med.* 8, 319–323
3 Hellerstein, M. (2002) HIV tropism and CD4$^+$ T-cell depletion. *Nat. Med.* 8, 537–538
4 Feinberg, M.B. *et al.* (2002) HIV tropism and CD4$^+$ T-cell depletion. *Nat. Med.* 8, 537
5 Gratzner, H.G. (1982) Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science* 218, 474–475
6 Hellerstein, M.K. and Neese, R.A. (1992) Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers. *Am. J. Physiol.* 263, E988–1001
7 Mohri, H. *et al.* (1998) Rapid turnover of T lymphocytes in SIV-infected rhesus macaques. *Science* 279, 1223–1227
8 Bonhoeffer, S. *et al.* (2000) Quantification of cell turnover kinetics using 5-bromo-2′-deoxyuridine. *J. Immunol.* 164, 5049–5054
9 Debacq, C. *et al.* (2002) Increased cell proliferation but not reduced cell death, induces lymphocytosis in bovine leukemia virus-infected sheep. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10048–10053
10 Mohri, H. *et al.* (2001) Increased turnover of T lymphocytes in HIV-1 infection and its reduction by anti-retroviral therapy. *J. Exp. Med.* 194, 1277–1287
11 Haynes, B.F. *et al.* (2000) The role of the thymus in immune reconstitution in aging, bone marrow transplantation and HIV-1 infection. *Annu. Rev. Immunol.* 18, 529–560
12 Ye, P. and Kirschner, D.E. (2002) Reevaluation of T-cell receptor excision circles as a measure of human recent thymic emigrants. *J. Immunol.* 168, 4968–4979
13 McLean, A.R. and Michie, C.A. (1995) *In vivo* estimates of division and death rates of human T lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* 92, 3707–3711
14 Michie, C.A. *et al.* (1992) Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature* 360, 264–265
15 Roederer, M. *et al.* (1997) Dynamics of fine T-cell subsets during HIV disease and after thymic ablation by mediastinal irradiation. *Semin. Immunol.* 9, 389–396
16 Rosenzweig, M. *et al.* (1998) Increased rates of CD4(+) and CD8(+) T-lymphocyte turnover in simian immunodeficiency virus-infected macaques. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6388–6393