Mathematical Modelling of the Interleukin-2 T-cell System: A Comparative Study of Approaches Based on Ordinary and Delay Differential Equations

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Cell proliferation and differentiation phenomena are key issues in immunology, tumour growth and cell biology. We study the kinetics of cell growth in the immune system using mathematical models formulated in terms of ordinary and delay differential equations. We study how the suitability of the mathematical models depends on the nature of the cell growth data and the types of differential equations by minimizing an objective function to give a best-fit parameterized solution. We show that mathematical models that incorporate a time-lag in the cell division phase are more consistent with certain reported data. They also allow various cell proliferation characteristics to be estimated directly, such as the average cell-doubling time and the rate of commitment of cells to cell division. Specifically, we study the interleukin-2-dependent cell division of phytohemagglutinin stimulated T-cells — the model of which can be considered to be a general model of cell growth. We also review the numerical techniques available for solving delay differential equations and calculating the least-squares best-fit parameterized solution.

Keywords: Cell proliferation, Interleukin-2, Mathematical modelling, Parameter estimation, Time-lag.

INTRODUCTION

An important problem in various branches of bioscience is the derivation of mathematical descriptions (models) of real-life phenomena that are quantitatively consistent with experimental observations. These models can then be used to provide feedback to researchers on the suitability of experimental data, and they in turn can help improve and refine the mathematical models. Thus the mathematical modelling complements the practical experimentation and vice versa. Mathematical modelling also provides a systematic way of organizing experimental data on the behaviour of biological systems at the cell level, the tissue level, the organ level and the 'whole human' level. In doing so, it provides the opportunity of improving both the understanding and prediction of biological

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phenomena. Thus, in order to allow experimentalists to contribute to the derivation and improvement of models, the advantages and disadvantages of the various modelling approaches need to be made clear.

The purpose of this paper is to compare two different approaches to formulating mathematical models for real-life phenomena, in particular for interleukin-2 (IL-2) T-cell growth. The first approach models the cell division using only ordinary differential equations (ODEs), whereas the second approach uses delay differential equations (DDEs) (which include ODEs as a special case). The comparison is achieved by modelling typical experimental cell growth data, and assessing the quality of the fit of the mathematical models to the data.

Cell growth, or cell proliferation, is a central topic in cell biology, immunology and tumour growth. Historically, ODEs have been used to model cell growth — this is mainly due to their mathematical simplicity and the long-standing availability of software for solving them. However, it is obvious that cell division, as well as cell differentiation and cell maturation, are not instantaneous processes but take a finite time to occur. In some cases the durations of the cell processes can be ignored but, in principle, they should be included in the model so that it is consistent with the cell growth kinetics. (Some experimental data has features that are consistent with there being a time-lag in the cell division phase.) When ODE models are used, the delays can be modelled indirectly, for example, by a special choice of parameter values, by introducing 'hidden' variables (so-called 'gearing up' functions; De Boer and Perelson, 1991), or by introducing intermediate phases into the cell division model. Thus, avoiding the explicit modelling of the delays yields a mathematically less complex model. However, it has been suggested (Bocharov and Romanyukha, 1994a, Marchuk, Romanyukha and Bocharov, 1991; and Morel, Kalagnanam and Morel, 1992) that a delay (or time-lag) in the cell division naturally implies the use of DDEs in the corresponding mathematical model.

In our recent work (Baker, Bocharov, Paul and Romanyukha, 1995; and Baker, Bocharov, and Paul

1997), we showed that a mathematical model of cell growth that incorporated a time-lag in the cell division phase provided both a qualitatively and quantitatively better fit to certain reported data than the classical exponential ODE growth model. In Baker, Bocharov, and Paul (1997), we analyzed three different patterns of cell growth using simple exponential growth and time-lag growth models. First we analyzed experimental data for the growth of pre-B-cells in different concentrations of fetal calf serum (Jonassen, Seglen and Stokke, 1994). The pre-B-cell growth data exhibit exponential growth and, in fact, the ODE and DDE models are equally consistent, both qualitatively and quantitatively. Next we analyzed the growth of fission yeast, using data that does not exhibit exponential growth (Moreno and Nurse, 1994). In this instance, there were significant qualitative and quantitative differences between the ODE and DDE models, with the DDE model proving to be substantially better than the ODE model. Additionally, the DDE model can provide direct estimates of (i) the cell-doubling time, (ii) the fraction of the cells that are dividing, (iii) the rate of commitment of cells to cell division and (iv) the initial distribution of cells in the cell cycle, whereas the ODE model only provides an indirect estimate of the culture-doubling time. Thus the use of DDEs in mathematical modelling permits a richer framework for analyzing real-life phenomena, as well as allowing parameters to be introduced in a scientifically meaningful manner.

The mathematical modelling of cell growth relies on the determination of the values of model parameters that provide the best-fit solution to experimental data. One aim of this paper is to highlight the availability of numerical software for solving DDEs, and for solving the least-squares best-fit parameter estimation problem.

Here, we analyze the growth of T-cells resulting from the interaction with cytokines. It has been suggested that the effect of exogenous IL-2 on the growth of phytohemagglutinin (PHA) stimulated T-cells is typical of cell growth in general (Cantrel and Smith, 1984). Thus, analyzing the kinetics of T-cell growth should provide insight into the dynamics of

more general mammalian cell growth. We use two models to describe T-cell growth, both having the same state variables and similar expressions for the rates of growth. The main difference between the models is how the cell cycle is represented — one model explicitly includes a time-lag in the cell division, representing the delay in the appearance of new cells, and the other does not. Thus the first model uses DDEs, and the other uses only ODEs.

The derivation of the mathematical models for IL-2 T-cell growth is discussed in some detail. The models are then compared, by fitting some typical experimental T-cell growth data to them. Finally, we discuss the numerical techniques used for solving the DDE models and the parameter estimation problem, highlighting a number of important issues that must be addressed.

MATHEMATICAL MODELS OF IL-2 T-CELL GROWTH

Background

It has been suggested that the growth of T-cells in response to polyclonal stimulation by PHA is typical of cell growth in general (Cantrel and Smith, 1984; Smith, 1988). Thus the growth characteristics of the IL-2 T-cell system are identical, for example, to those of bacteria, yeasts, protozoa and mammalian cells. Therefore, analyzing the kinetics of T-cell growth may provide insight into the dynamics of more general cell growth. The models of IL-2 T-cell growth in this section are based on the following observations on the growth of IL-2-dependent lectin-activated T-cells (summarized from Cantrel and Smith, 1983; Cantrel and Smith, 1984; Smith, 1988).

- Antigenic stimulation of T-cell receptors induces virgin or naive T-cells to progress from the G_0-phase to the G_1-phase of the cell cycle and to exhibit high affinity IL-2 binding sites.
- Following the antigenic stimulation of T-cells, the high affinity IL-2 receptors (IL-2r) and low affinity IL-2r occur in the ratio 1:10 on the cell surface.

- It is thought that only the high affinity IL-2r are able to bind IL-2 at physiological concentrations and internalize it, so as to initiate T-cell growth.
- Further progression of an activated T-cell from the G_{1a}-phase through the G_{1b}-, S-, G_2- and M-phases of the cell cycle is promoted by the interaction of IL-2 with IL-2r on the surface of the T-cell.
- IL-2r appear asynchronously in the T-cells of PHA-activated human peripheral blood.
- T-cell populations exhibit a marked diversity in the expression of IL-2r, although there is a correlation between the IL-2r density and the rate of T-cell growth.
- The accumulation of IL-2r by a cell is a gradual and asynchronous process, and precedes the commitment of the cell to cell division.
- The IL-2r density amongst activated T-cells can vary by a factor of 1000, and has a log-normal distribution — similar to the variation in the duration of the cell cycle (Moreno and Nurse, 1994).
- The continued progression of a T-cell through the phases of the cell cycle depends on the concentration of available IL-2 and the duration of the interaction between IL-2 and IL-2r.
- The IL-2 log(dose)-response curve is sigmoid-shaped, reflecting the saturation effect of too high a concentration of IL-2 on T-cell growth.
- The duration of the cell division of IL-2-stimulated T-cells represents the time taken for a T-cell to pass from the G_{1b}-phase through to the M-phase.

In modelling *in vitro* T-cell growth, we introduce the following time-dependent variables:

$I_2(t)$: concentration of exogenous IL-2,

$T_A(t)$: concentration of PHA-activated T-cells expressing high affinity IL-2r,

$T_D(t)$: concentration of IL-2-stimulated T-cells entering the cell division cycle,

$T_R(t)$: concentration of 'resting' T-cells with no binding sites to IL-2.

However, in practice, the experimental data often corresponds to the concentration of the whole T-cell population, namely $T_A(t) + T_D(t) + T_R(t)$.

The Mathematical Models

The equations for describing IL-2 T-cell growth are based on the Law of Mass Action, and take into account the effects of IL-2 saturation and the time-lag in the cell cycle. The derivation of both models is also influenced by our previous modelling experience (Bocharov and Romanyukha, 1994a; Marchuk, Romanyukha and Bocharov, 1991; Sidorov and Romanyukha, 1993).

A *time-lag model*

(i) The equation for the kinetics of IL-2 is

$$I_2'(t) = -\alpha_{I_2}I_2(t) - n_{I_2T}b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}T_A(t). \tag{1}$$

The two processes responsible for the decrease in IL-2 — natural death and internalization by T-cells expressing IL-2r — are both modelled.

(ii) The equation for activated T-cells expressing IL-2r is

$$T_A'(t) = \rho b_{TI_2}\frac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1}$$
$$\times T_A(t - \tau_D) - b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}$$
$$\times T_A(t) - \alpha_{AR}T_A(t) \tag{2}$$

The processes modelled are the creation of T-cells by cell division, the progression of IL-2-stimulated T-cells into the cell division cycle, and the decline in IL-2r expression due to its transient nature (Cantrel and Smith, 1983; Cantrel and Smith, 1984). The model used for the appearance of new cells and the progression of activated T-cells into the cell division cycle is based on a model of the antiviral immune response (Bocharov and Romanyukha, 1994a; Marchuk, Romanyukha and Bocharov, 1991; and Sidorov and Romanyukha, 1993).

(iii) The number of T-cells that are currently in the cell division cycle is determined by

$$T_D'(t) = b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}T_A(t) - b_{TI_2}$$
$$\times \frac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1}T_A(t - \tau_D), \tag{3}$$

which follows directly from (2).

(iv) Finally, the equation modelling 'resting' T-cells with no binding sites to IL-2 is

$$T_R'(t) = \alpha_{AR}T_A(t) - \alpha_R T_R(t).$$

The two factors that affect the number of 'resting' T-cells are the return of activated T-cells to the resting phase and natural death.

Parameters in the time-lag model

As we have already mentioned, one advantage of using a DDE model over an ODE model is that the parameters in the DDE model usually have a direct biological interpretation. The time-lag model has the following parameters:

α_{I_2}: decay rate of IL-2 in the medium, ≈ 0 molec./hr in fetal calf serum.

n_{I_2T}: number of IL-2 molecules internalized by T-cells via IL-2r, 2000–5000 per T-cell.

b_{TI_2}: rate of commitment of T-cells to cell division, 10^{-12}–10^{-11} ml/(molec. × hr).

I_2^*: saturation concentration for IL-2, 6×10^{10} molec./ml.

ρ: number of cells produced when a T-cell divides, 2.

τ_D: duration of the cell division cycle, 8–24 hrs.

α_{AR}: decay rate in IL-2 reactivity of activated T-cells, 0.02 hr^{-1}.

α_R: decay rate in the non-cycling T-cell population, 0.01–0.04 hr^{-1}.

The initial estimates for the values of the parameters were derived using data from Cantrel and Smith (1983), Cantrel and Smith (1984), Ishida *et al.* (1987), Sidorov and Romanyukha (1993), and Smith (1988). The time-lag model also requires initial functions for $T_A(t)$ and for $I_2(t)$ to be specified, representing the heterogeneity of T-cells expressing IL-2r and the IL-2 concentration before the start of

the experiment, respectively (see Baker, Bocharov and Paul, 1997).

The 'instantaneous' (ODE) model

Equations (2) and (3) can be rewritten without the delay τ_D, yielding the following system of ODEs for modelling IL-2 T-cell growth:

(i) The equation for the kinetics of IL-2 is unchanged,

$$I_2'(t) = -\alpha_{I_2}I_2(t) - n_{I_2T}b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}T_A(t).$$

(ii) With no explicit delay, the equation for activated T-cells expressing IL-2r becomes

$$T_A'(t) = \rho b_D T_D(t) - b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}$$
$$\times T_A(t) - \alpha_{AR}T_A(t). \qquad (4)$$

(iii) The equation for the number of T-cells that are currently in the cell division cycle follows directly from the equation for $T_A'(t)$ (as it did in the DDE case),

$$T_D'(t) = b_{TI_2}\frac{I_2(t)}{I_2(t)/I_2^* + 1}T_A(t) - b_D T_D(t). \qquad (5)$$

(iv) The equation modelling 'resting' T-cells with no binding sites to IL-2r remains unchanged,

$$T_R'(t) = \alpha_{AR}T_A(t) - \alpha_R T_R(t).$$

However, it should be noted that the ODE formulation of the DDE model (above) is not unique. For example, equations (4) and (5) may be combined to give a single equation,

$$T_{A+D}'(t) = (\rho - 1)b_D T_{A+D}(t) - \alpha_{AR}T_{A+D}(t)$$

with the single parameter b_D characterizing the rate of growth of T-cells.

Parameters in the 'instantaneous' model

There is a direct correspondence between most of the parameters in the ODE model and those in the DDE model (above), the two exceptions are the new parameter b_D and the parameter b_{TI_2} each of which now has a different interpretation.

b_D: rate of cell division.

b_{TI_2}: rate of cells entering the cell division cycle.

Both of these parameters have a less well-defined biological interpretation compared to those in the time-lag model. In the time-lag model, the cell division cycle is naturally modelled by two parameters — the rate of commitment of T-cells to cell division, b_{TI_2}, and the duration of the cell division cycle, τ_D.

Comparing Various Models of IL-2 T-cell Growth

Several mathematical models that include equations for modelling IL-2 T-cell growth have recently been proposed by McLean (1992), Morel, Kalagnanam and Morel (1992), and Sidorov and Romanyukha (1993). However, the comparatively limited experience in the quantitative modelling of IL-2 T-cell growth makes it difficult to provide definitive comparisons between the various models. Thus it is difficult to determine which equations are most consistent with the available data, although it is still useful to examine the structure of each of the various models. We examine three of the current models: (a) by McLean (1992), (b) by Morel, Kalagnanam and Morel (1992), and (c) by the authors. In each case, the same notation is used in order to allow easier comparison. (There is also a more complex model of T-cell growth by Sidorov and Romanyukha (1993) that includes equations for IL-2 T-cell growth. It is closer to our model than the models of McLean and of Morel et al., the main difference is that the Sidorov and Romanyukha model does not take into account the effects of IL-2 saturation.)

- Equations for exogenous IL-2

  (a) $I_2'(t) = \text{Source}_{I_2} - \psi I_2(t) - \beta I_2(t)T_A(t)$

  (b) $I_2'(t) = \text{Source}_{I_2} - p_2 I_2(t)(T_R(t) + T_A(t) + T_D(t))$

  (c) $I_2'(t) = \text{Source}_{I_2} - \alpha_{I_2}I_2(t) - n_{I_2T}b_{TI_2}$
  $$\times \frac{I_2(t)}{I_2(t)/I_2^* + 1}T_A(t)$$

- Equations for activated T-cells expressing IL-2r

  (a) $T'_A(t) = \text{Source}_{T_A} - \rho \dfrac{T_A(t)}{T_A(t)/\xi + 1} I_2(t)$

  $\qquad - \mu T_A(t)$

  (b) $T'_A(t) = T'_R(t - T_2) - T'_D(t)$.

  (c) $T'_A(t) = \rho b_{TI_2} \dfrac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1} T_A(t - \tau_D)$

  $\qquad - b_{TI_2} \dfrac{I_2(t)}{I_2(t)/I_2^* + 1} T_A(t) - \alpha_{AR} T_A(t).$

- Equations for T-cells currently in the cell division cycle

  (a) N/A

  (b) $T'_D(t) = b_2 I_2(t) T_A(t) - T'_D(t - T_1)$

  (c) $T'_D(t) = b_{TI_2} \dfrac{I_2(t)}{I_2(t)/I_2^* + 1} T_A(t) - b_{TI_2}$

  $\qquad \times \dfrac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1} T_A(t - \tau_D).$

- Equations for resting T-cells with no binding sites to IL-2r

  (a) $T'_R(t) = \text{Source}_{T_R} - \mu T_R(t)$

  (b) $T'_R(t) = 2T'_D(t - T_1) - T'_A(t)$

  (c) $T'_R(t) = \alpha_{AR} T_A(t) - \alpha_R T_R(t).$

It is clear that there is no unique mathematical model of IL-2 T-cell growth. This is partly due to the fact that there is no systematic method for formulating models of cell growth. A key goal when formulating a model should be an appropriate balance between the available experimental data and the specification of the interactions between cells. However, there seems to be no generally accepted objective criterion for what constitutes quantitative consistency of a mathematical model with experimental data.

QUANTITATIVE MODELLING OF EXPERIMENTAL DATA

IL-2 T-cell Growth: G_1 – phase $\rightarrow$ S-phase

T-cells that had been synchronized by being grown in a low concentration of IL-2 for 2 weeks had a receptor-saturating concentration of IL-2 added. The number of T-cells entering the S-phase of the cell cycle was determined by adding tritiated thymidine ([³H]TdR). The amount of [³H]TdR incorporated by the T-cells is indirectly related to the number of T-cells entering the S-phase, and it is therefore necessary to relate the experimental data to the variable $T_D(t)$. This may be achieved by a process of linear regression using data from Figure 3 in Smith (1988). The resulting experimental data on the initial phase of PHA-blast growth can then be used to improve the estimates of b_{TI_2} and τ_S, where τ_S is the time taken for a T-cell to progress from the G_1-phase to the S-phase. The following initial values for the model were used:

$I_2(0) = 6 \times 10^{10}$ molec./ml,

$T_A(0) = 5 \times 10^4$ cells/ml,

$T_D(0) = 0$ cells/ml,

$T_R(0) = 0$ cells/ml.

The initial function for $I_2(t)$ corresponds to the concentration of IL-2 in the cell culture before the start of the experiment. The initial function for $T_A(t)$ represents the initial heterogeneity in the T-cells expressing IL-2r and, indirectly, the progress of T-cells already in transition from the G_1-phase to the S-phase of the cell cycle. The initial heterogeneity in the T-cells expressing IL-2r implies that, initially, the T-cells grow at a uniform rate. Thus the corresponding initial functions are specified as

$\left.\begin{array}{l} I_2(t) = 0 \text{ molec./ml} \\ T_A(t) = 5 \times 10^4 \text{ cells/ml} \end{array}\right\}$ for $t \in [-\tau_D, 0]$.

By explicitly modelling τ_S, the equations for $T'_A(t)$ and $T'_D(t)$ need to be rewritten:

$T'_A(t) = \rho b_{TI_2} \dfrac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1}$

$\qquad \times T_A(t - \tau_D) - b_{TI_2} \dfrac{I_2(t - \tau_S)}{I_2(t - \tau_S)/I_2^* + 1}$

$\qquad \times T_A(t - \tau_S) - \alpha_{AR} T_A(t),$

$T'_D(t) = b_{TI_2} \dfrac{I_2(t - \tau_S)}{I_2(t - \tau_S)/I_2^* + 1} T_A(t - \tau_S) - b_{TI_2}$

$\qquad \times \dfrac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1} T_A(t - \tau_D),$

where the duration of the cell cycle $\tau_D = \tau_S + \tau_{G_1}$ – with τ_{G_1} being the time taken for a T-cell to progress
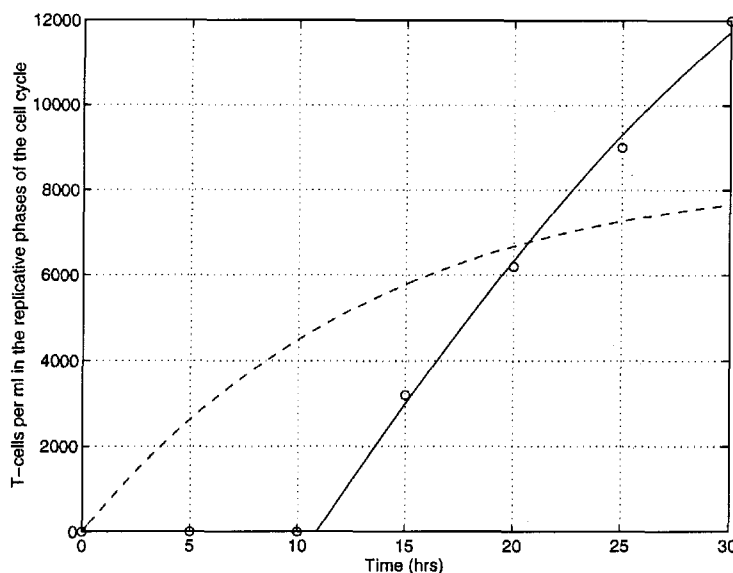
FIGURE 1 IL-2 T-cell growth — transition from G_1 — phase → S-phase. The least-squares best-fit solutions of the ODE model (dashed line) and the best-fit DDE model (solid line) plotted against the experimental data (O).

from the S-phase of the cell cycle through to the G_1-phase.

Due to the sparsity of data in relation to the number of parameters in the mathematical models, it is necessary to fix some of the parameter values using information from Cantrel and Smith (1983), Cantrel and Smith (1984) and Smith (1988).

$\alpha_{I_2} = 0 \text{ hr}^{-1}$

$n_{I_2T} = 2000$

$I_2^* = 6 \times 10^{10} \text{ molec./ml}$

$\tau_S = 10 \text{ hrs}$

$\alpha_{AR} = 0.02 \text{ hr}^{-1}$

$\rho = 2$

$\tau_{G_1} = 18 \text{ hrs}$

$\alpha_R = 0.01 \text{ hr}^{-1}$

$b_D = 1/\tau_D$

Using experimental data from Smith (1988),

TABLE I Experimental data for IL-2 T-cell growth: G_1-phase → S-phase

Time (hrs)	0.0	5.0	10.0	15.0	20.0	25.0	30.0
$T_D(t) \times 10^3$	0.0	0.0	0.0	3.2	6.2	9.0	12.0

the least-squares best-fit solutions correspond to the following parameter values:

TABLE II Best-fit parameter values for IL-2 T-cell growth: G_1-phase → S-phase

ODE model		DDE models			
$b_{T_{I_2}}$ (ml/(molec. ×hr))	||Error||₂	$b_{T_{I_2}}$ (ml/(molec. ×hr))	τ_S (hrs)	τ_{G_1} (hrs)	||Error||₂
4.1×10^{-13}	7480	5.1×10^{-13}	–	–	1817
–	–	5.3×10^{-13}	11.5	–	733
–	–	5.1×10^{-13}	10.9	20.4	466

It is clear from both Figure 1 and Table II that there is both a significant qualitative and quantitative difference between the best-fit solutions corresponding to the two types of model.* The quantitative differences are apparent from the size of the least-squares objective function $\|\|Error\|\|_2$. However, because the initial functions are constants and the maximum data point occurs at $t = 30$, the value of the objective function is constant for $\tau_{G_1} \geq 30 - \tau_S$. This highlights the crucial need for experimental data to be given for a sufficiently long

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*For the ODE model, the nature of the data suggests that it might be advantageous to ignore the first two data points and solve the model for $t \geq 10$ using the same initial data, the resulting best-fit parameter value is $b_{T_{I_2}} = 6.9 \times 10^{-13}$ with $\|\|Error\|\|_2 = 2062$.

period of time, especially when the duration of the cell cycle is being estimated *directly* (as in the DDE case).

Growth of PHA-blasts Against Exogenous IL-2

PHA-blasts were cultured at 5×10^5 cells/ml with 1U/ml of IL-2r, and the number of viable cells in the culture was counted every 24 hrs. The average number of high affinity IL-2r on the PHA-blasts (from normal subjects) was about 4755 per cell. Using experimental data for the growth kinetics of PHA-blasts from Figure 5 in Ishida *et al.* (1987), estimates for the values of b_{TI_2} and τ_D were improved by considering the complete cell cycle for T-cells. The initial values used were

$I_2(0) = 2 \times 10^{10}$ molec./ml,
$T_A(0) = 3.8 \times 10^5$ cells/ml,
$T_D(0) = 0$ cells/ml,
$T_R(0) = 1.2 \times 10^5$ cells/ml,

with the following initial functions

$\left.\begin{array}{l} I_2(t) = 0 \text{ molec./ml} \\ T_A(t) = 5 \times 10^5 \text{ cells/ml} \end{array}\right\}$ for $t \in [-\tau_D, 0]$,

which corresponds to IL-2 being added to the culture at the start of the experiment. The observable data corresponds to the total number of viable cells in the culture, $T_V(t) = T_A(t) + T_D(t) + T_R(t)$. From above the DDE and ODE models for $T_V'(t)$ are

$$T_V'(t) = (\rho - 1)b_{TI_2}\frac{I_2(t - \tau_D)}{I_2(t - \tau_D)/I_2^* + 1}$$
$$\times T_A(t - \tau_D) - \alpha_R T_R(t)$$

and

$$T_V'(t) = (\rho - 1)b_D T_D(t) - \alpha_R T_R(t),$$

respectively. Again, due to the sparsity of data, a number of parameter values are fixed based on values obtained from Cantrel and Smith (1983), Cantrel and Smith (1984), Ishida *et al.* (1987), and Smith (1988).

$\alpha_{I_2} = 0 \text{ hr}^{-1}$
$n_{I_2T} = 4755$
$b_D = 1/\tau_D$
$I_2^* = 6 \times 10^{10}$ molec./ml
$\tau_D = 8 \text{ hrs}$
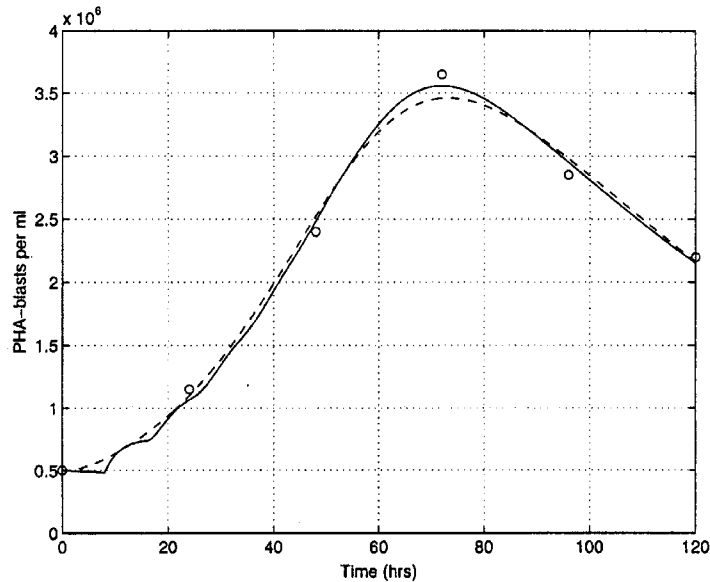$\alpha_{AR} = 0.02 \text{ hr}^{-1}$
$\rho = 2$

FIGURE 2   PHA-blast growth in normal subjects. The least-squares best-fit solutions of the ODE model (dashed line) and the best-fit parameter DDE model (solid line) plotted against the experimental data (O).

Using data from Ishida *et al.* (1987),

TABLE III   Experimental data for growth of PHA-blasts

Time (hrs)	0.0	24.0	48.0	72.0	96.0	120.0
$T_V(t) \times 10^6$	0.50	1.15	2.40	3.65	2.85	2.20

we obtained the following least-squares best-fit parameter values:

TABLE IV   Best-fit parameter values for growth of PHA-blasts

ODE model		
b_{TI_2}	α_R (hr^{-1})	$\|\|Error\|\|_2$
1.0×10^{-11}	3.5×10^{-2}	2.7×10^5
–	–	–
–	–	–

DDE models				
b_{TI_2}	α_R (hr^{-1})	τ_D (hrs)	α_{AR} (hr^{-1})	$\|\|Error\|\|_2$
1.2×10^{-11}	3.7×10^{-2}	–	–	2.4×10^5
9.6×10^{-12}	3.4×10^{-2}	6.6	–	2.2×10^5
1.8×10^{-11}	1.5×10^{-2}	–	6.6×10^{-2}	1.8×10^5

The solutions in Figure 2 corresponding to the best-fit parameter values of the ODE and DDE models given in Table IV clearly exhibit similar qualitative and quantitative types of cell growth. However, the sparsity of the data prevents any further conclusions being drawn.

NUMERICAL SOFTWARE FOR ANALYZING DDE MODELS

Numerical Solution of DDEs

The traditional approach to solving DDEs numerically has been to adapt an ODE solver so that it stores the past solution. However, in adapting an ODE solver, there are several points that should be borne in mind (Baker, Paul and Willé, 1995):

- the provision of a suitable — robust, but reasonably cheap — continuous extension (or dense output) for evaluating the delayed solution terms;
- the existence of derivative discontinuities in the solution that propagate forward in time;

- the possibility of a vanishing delay, when $\tau(t) \rightarrow 0$, and its impact on codes that use explicit solution methods.

The correct choice of continuous extension is important, because its order of accuracy should be the same as the order of the underlying ODE method in order to maintain the asymptotic correctness of the error estimator. Additionally, the continuous extension should be 'stable', in the sense that it does not adversely affect the numerical stability properties of the solution. For DDEs that only have non-decreasing delayed arguments, the solution becomes smoother as time increases, so that 'eventually' the existence of derivative discontinuities can be ignored.

There are currently a number of general purpose codes for solving initial value problems for DDEs. An important feature of such codes is that they aim to produce a solution to within a given accuracy for a wide range of requested tolerances. Paul (1995) has developed such a code based on the successful Dormand and Prince fifth-order Runge–Kutta method for ODEs and the fifth-order Hermite interpolant due to Shampine. The resulting code is uniformly fifth-order accurate for ODEs, DDEs and neutral differential equations (where the derivative additionally depends on delayed derivative values)[†].

Parameter Estimation for DDEs

The task of parameter estimation is one of minimizing an objective function $\Phi(p)$ based on the unknown parameters p and sample data. In the case of parameter estimation for DDEs, this can include estimating parameters in the DDE and the initial values (as in the ODE case), but additionally estimating the position of the initial point, the initial functions and the delayed arguments.

Criteria for best-fit parameter values

The typical objective function is the classical least-squares (LSQ) function. In the LSQ approach, the

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[†] The code is available for non-commercial purposes by E-mailing chris@ma.man.ac.uk.

values of the unknown parameters are estimated by minimizing the sum of the squared residuals,

$$\Phi(wf) = \sum_{i,j} w_{ij}(y^i_{\text{obs}_j} - y^i(t_j, wf))^2,$$

where the $\{w_{ij}\}$ are weights (possibly related to the accuracy of the data points), $y^i_{\text{obs}_j}$ is the jth experimental datum for the ith component of the model, and $y^i(t_j, \mathrm{p})$ is the value of the ith component of the model at the jth data point corresponding to the parameter values p. A significant feature of the LSQ approach is that a small relative change in large data values can be unduly weighted. For example, a 1% change in the value 100 leads to a squared residual of 1, whereas a 1% change in the value 1,000,000 leads to a squared residual of 10,000,000.

This aspect of the LSQ approach can be critical when modelling the immune system, because a typical set of data can have a large variation in scale but with each datum being equally significant. For these sets of data, the log least-squares (LLSQ) approach seems to be more appropriate for determining the best-fit parameter values (Bocharov and Romanyukha 1994b; Morel, Kalagnanam and Morel, 1992). The corresponding objective function is

$$\Phi(\mathrm{p}) = \sum_{i,j} w_{ij}(\log |y^i_{\text{obs}_j}| - \log |y^i(t_j, \mathrm{p})|)^2.$$

For a mathematical model formulated in terms of ODEs or DDEs, the LSQ approach leads to a nonlinear minimization problem. However it can be shown that the overall degree on non-linearity of the objective function $\Phi(\mathrm{p})$ for the LLSQ approach is less than that of the LSQ approach for the same problem (Bocharov and Romanyukha 1994a). Thus, an appropriate choice of objective function is an important factor in determining the ease of solving the parameter estimation problem, since the choice of objective function strongly affects the non-linearity of the minimization problem.

Numerical techniques for parameter estimation

Given a set of (experimental) data, the technique for finding the best-fit parameter values for a given mathematical model and objective function involves solving the model equations using the current values of the parameters. The parameter values are then adjusted (by the minimization routine) so as to reduce the value of the objective function. However, in order to find the *global* best-fit parameter values, the initial estimate of the parameter values must be sufficiently close to the global minimum. Thus good starting estimates for the parameter values can be of great assistance, both in speeding up the minimization process and finding the global minimum. Such estimates can sometimes be obtained by a sequential process of finding the best-fit parameter values for subsets of the data, where the subsets are usually obtained by subdividing the observation interval. As the size of the subinterval increases, the best-fit parameter values can be improved in a step-by-step manner. This approach can be very efficient for parameter estimation in some immune response models (Bocharov and Romanyukha, 1994a; Bocharov and Romanyukha, 1994b; Marchuk, Romanyukha and Bocharov, 1991).

There are a number of general purpose least-squares minimization routines available, for example, E04UPF in the NAg library, LMDIF from NETLIB and fmins in MATLAB. (In this paper, we used both the *unconstrained* minimization code LMDIF, and the *constrained* minimization code E04UPF.) However, there are a number of points that should be noted:

- First, the model solution values $\{y^i(t_j, \mathrm{p})\}$ are obtained numerically. Thus the actual values used in the objective function are perturbed solution values $y^i(t_j, \mathrm{p}) + \delta_{ij}$, where δ_{ij} is dependent on the user-requested tolerance in the ODE/DDE solver. This limited accuracy of the solution values must be accounted for in the minimization process, and this can ultimately be achieved by specifying the correct number of digits in the value of the objective function.

- If the minimization process uses numerical approximations to either the partial derivatives or the Jacobian of the objective function, then the effect of the limited accuracy of the model

solution values must be assessed. In particular, it is usually implied that the accuracy of the model solution does not need to be greater than that of the data. In fact, the model solution must be obtained to greater accuracy if the convergence rates of derivative-based minimization methods are to be realized.

- One of the main assumptions in minimization theory is on the smoothness of the objective function. It is usually assumed that the objective function has sufficiently smooth derivatives *everywhere*. However, in the case of parameter estimation for DDEs, Baker and Paul (1995) showed that the objective function (6) can be both discontinuous and have discontinuous partial derivatives *anywhere*. This can seriously affect the reliability and robustness of minimization codes that rely on having a smooth objective function.

- In terms of efficiency, it may be advantageous to use a combination of minimization methods. The initial estimate of the parameter values can first be improved by a computationally cheap method, such as a derivative-free direct search method. The resulting estimate of the best-fit parameter values can then be improved using a computationally expensive but rapidly converging method, such as a Newton-based method.

- The convergence of a minimization method can be improved by specifying lower and/or upper bounds on the values of the model parameters (based on *a priori* information). In doing so, the computational effects of the variations in scale in the ranges of the parameter values can be reduced by rescaling the parameters to be of the same order of magnitude.

CONCLUSIONS

The main objective of this paper is to demonstrate that some real-life phenomena are better modelled, in terms of qualitative and quantitative consistency with experimental data, by mathematical models that include explicit time-lags. In doing so, we hope to convince modellers that they should not restrict themselves to using only ODEs, because efficient and reliable codes for solving DDEs are available. Additionally, because DDEs model real-life phenomena more precisely, they allow more biologically meaningful parameters to be modelled directly (see Baker, Bocharov, and Paul 1997). Our work has been based on:

- well-founded numerical techniques for solving DDEs and for minimization, and

- analysis of various mathematical models used in modelling cell division.

Using this expertise, we compared the qualitative and quantitative consistency of two basic models (one with time-lags and the other without) for some typical experimental data on the growth of T-cells. In our view, T-cell growth has features in common with many other biological systems, such as population growth, and immunological and epidemiological phenomena (Marchuk, Romanyukha and Bocharov, 1991). In consequence, our study here of T-cell growth, and of fission yeast in Baker, Bocharov, and Paul (1997), might be of wider interest to experimentalists working in cell growth and differentiation phenomena. Indeed, the delays that appear in DDE models are often directly measurable and explicitly controllable biological parameters. However, it should be noted that ODE modelling compliments the DDE modelling approach, in that the best-fit parameter values obtained from an ODE model can be used as initial estimates for the corresponding parameter values in the DDE models.

The coverage of DDEs in the literature is now quite extensive, both from the mathematical perspective (Baker, Paul and Willé, 1995), and the modelling perspective (Banks, Burns and Cliff, 1981; Epstein, 1992). The DDEs used in this paper are of the simplest kind, having constant time-lags, and represent the simplest type of integro-differential equations (IDEs). Other IDEs can provide even greater opportunity for modelling hereditary effects on the rate of cell growth, however they are typically computationally more expensive to solve. For example, the term in (1) representing the reduction in IL-2 due to internalisation by T-cells might more

naturally be given as an integral term, corresponding to the *gradual* reduction in IL-2.

Finally, in order for experimentalists to be able to contribute to the formulation and testing of mathematical models, they need to know what types of equation are feasible. We hope that the results presented in this paper demonstrate convincingly that there are distinct advantages to using DDEs in some cases, and that the increased mathematical complexity of DDE models presents no further difficulty numerically than ODE models.

Acknowledgments

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