Review Paper

An introduction to lymphocyte and viral dynamics: the power and limitations of mathematical analysis

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Mathematics is a useful tool in the analysis and understanding of population dynamic aspects of the immune response. However, the power of mathematical modelling in immunology is frequently limited by the shortage of experimental data. Here, we review the contribution of mathematics to two areas of immunology. We highlight the problem caused by lack of knowledge of the system, which can greatly restrict the use of mathematics and lead to errors caused by model-specific results.

Keywords: mathematical model; lymphocyte dynamics; theoretical immunology

1. INTRODUCTION

Over the past 15 years, mathematics has been increasingly applied to help understand the complex dynamics of the immune system and its response to viral infection. The contribution of mathematics to immunology is most apparent in HIV-1 research, where the large volume of experimental data available has made the use of mathematics more practicable. However, even in a well-studied system such as HIV-1 the application of mathematics is still severely hampered by ignorance of the system.

Here, we illustrate the application of mathematical modelling to immunology with two specific examples:

- (i) the calculation of the turnover rate of specific lymphocyte populations in uninfected subjects and in those with persistent viral infections such as HIV-1; and
- (ii) elucidation of the roles of cytotoxic T lymphocytes (CTLs) in persistent infection with the human leukaemia virus, HTLV-I.

In each case, the utility of mathematical modelling is in clarifying the hypothesis under test, finding the smallest number of factors necessary to explain the observations, and suggesting means of testing the hypothesis and analysing and interpreting the experimental results.

We discuss the problems that undermine the application of mathematics to immunology and suggest possible ways in which these problems can be reduced.

2. QUANTIFYING LYMPHOCYTE AND VIRAL DYNAMICS

One of the major contributions of mathematics to immunology has been in the quantification of lymphocyte and viral dynamics. In healthy individuals the sizes of a number of lymphocyte subpopulations are approximately constant or change only very slowly over time. Similarly,

in a number of chronic viral infections such as HIV-1 and HTLV-I, the viral load and the immune response to the virus are also approximately constant over long periods of time. It is not possible to infer lymphocyte turnover rates simply by observing this steady-state level. Instead, it is necessary either to perturb the steady state, for example by antiviral therapy, or to mark lymphocytes by adding a label that is not at steady state, such as BrdU, tritiated thymidine or deuterated glucose. In either case the subsequent *in vivo* dynamics can be very complex and mathematical modelling is a vital tool in interpreting such dynamics.

The quantification of lymphocyte dynamics by labelling experiments can be affected by two main variables—the labelling protocol (the type of label used and the length of the labelling period) and the mathematical model used to interpret the data. Experiments using a label that marks cells randomly (e.g. carboxy-fluorescein diacetate succinimidyl ester (CFSE), chromosome damage) would be expected to give very different death-rate estimates from experiments that label only dividing cells (e.g. BrdU, deuterated glucose) since the cell population examined is very different. Furthermore, if a dye that labels only dividing cells is used, short labelling periods will give different results from long labelling periods (since the former will be biased towards rapidly proliferating cells and the latter towards more slowly proliferating cells). The choice of mathematical model and the assumptions inherent in that model also affect both the kinetics estimated and the interpretation of those kinetics (Asquith *et al.* 2002; De Boer *et al.* 2003). For instance some models of BrdU or deuterated-glucose labelling partition label between a (partly) labelled source and dividing cells (Mohri *et al.* 1998; Bonhoeffer *et al.* 2000). Such models will give lower proliferation rates than models in which all label is assumed to arise from the division of cells and allowed to contribute to the estimated proliferation rate (Ribeiro *et al.* 2002; Debacq *et al.* 2002).

Some of the first experiments to estimate the *in vivo* kinetics of human lymphocytes studied patients who had recently received radiotherapy (Michie *et al.* 1992; McLean & Michie 1995). The resulting damage to the

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lymphocyte chromosomes was measured and modelled to estimate the lifespans of 'naive' (CD45RA⁺) and 'memory' (CD45RO⁺) T lymphocytes. Surprisingly, it was shown that primed memory cells divide much more rapidly (once every 22 weeks) than unprimed naive cells (once every 3.5 years). This changed the view of memory T cells—which were previously widely considered to be long-lived terminally differentiated resting cells—influencing not only basic perceptions of the maintenance of immune memory but also more applied areas such as vaccine design.

The same group also used modelling of chromosome damage to demonstrate that CD45RO⁺ cells can revert to a CD45RA⁺ phenotype, challenging the prevalent notion that there is a straightforward association between CD45 isoform and (foreign) antigen experience (Michie *et al.* 1992).

The behaviour of naive and memory T cells was further studied in a paper by Veiga-Fernandes *et al.* (2000). Here, the authors used mathematical techniques to analyse CFSE labelling of murine lymphocytes with the aim of comparing the *in vivo* responses of memory and naive CD8⁺ T cells to their cognate antigen. They found that, on meeting the antigen, memory T cells started to divide after a shorter lag time, divided at a faster rate and were lost at a lower rate than naive T cells. These observations confirmed that the difference between a primary and a secondary T-cell response is not simply caused by the number of cells that recognize the antigen but also by qualitative differences between memory and naive cells.

Both of these studies used labels that mark the population randomly (radiation damage and CFSE); such labels allow a more straightforward interpretation than labels that selectively mark proliferating cells. However, both of these labelling techniques have their drawbacks. Lymphocyte dynamics are likely to be seriously altered by radiotherapy and possibly by the condition necessitating therapy, making it difficult to infer normal dynamics from such studies. CFSE causes much less disturbance to the system, provided that it is directly injected rather than used to label cells *ex vivo* that are then transferred back to a host, but it is unsuitable for use in humans. The recent development (Hellerstein 1999) of a stable non-toxic label suitable for use in humans, deuterated glucose ([²H]glu), has enabled the study of *in vivo* lymphocyte dynamics in humans.

Much of the application of the [²H]glu labelling technique has focused on lymphocyte dynamics in HIV-1 seropositive subjects. In several important studies of HIV-1 infection, the proliferation rate and the death rate (Mohri *et al.* 2001) or alternatively the turnover rate (where the proliferation rate and the death rate are assumed to be equal; Hellerstein *et al.* 1999; McCune *et al.* 2000) of CD4⁺ and CD8⁺ T lymphocytes have been estimated. All the studies report an elevated proliferation rate of both CD4⁺ and CD8⁺ cells in HIV-1 infected subjects. However, the studies disagree on the impact of antiretroviral therapy. Work by Hellerstein *et al.* (1999) shows an *increase* in the turnover rate of CD4⁺ and CD8⁺ cells in subjects receiving highly active antiretroviral therapy (HAART), suggesting that a block in lymphocyte production may be a major cause of CD4⁺ cell depletion in HIV. Conversely, Mohri *et al.* (2001) clearly show that

the proliferation and death rates in both the CD4⁺ and CD8⁺ populations are *decreased* by HAART, suggesting that increased CD4⁺ cell destruction rather than decreased production causes CD4⁺ lymphopenia. These contradictory results could arise for a number of reasons, two of the most likely being differences in the patient groups studied and the mathematical models used. In the work by Hellerstein *et al.* (1999) the untreated and the HAART-treated groups may not be adequately matched; in the experiment by Mohri *et al.* (2001) this problem is overcome by studying the same group of patients before and after therapy. However, it seems unlikely that such a mismatch of the untreated and HAART-treated groups of Hellerstein *et al.* (1999) would actually cause a significant increase in turnover rates following HAART. Another possible factor contributing to the discrepancy may be the mathematical model used to interpret the labelling data. The two studies use very different models: Hellerstein *et al.* (1999) assume that the proliferation rate and the death rate of cells are equal, which Mohri *et al.* (2001) explicitly show to be untrue in their sample. The models used by Mohri *et al.* (2001) also introduce a large 'source' of mainly unlabelled cells. It has been argued (Asquith *et al.* 2002) that there is no physiological correlate of such a 'source' and that its use in models to analyse labelling data is unnecessary and could lead to the misinterpretation of data. It is therefore possible that the models used by both groups contain (different) flawed assumptions and that this may, at least in part, explain the discrepancies in their results.

The dependence of the results on the mathematical model used is a worrying aspect of nearly all mathematical modelling in immunology. Although the interpretation of labelling data is susceptible to model dependency as illustrated herein, this is much less of a problem than in many other areas of mathematical immunology. This is because, provided that the system is in a steady state, the parameters estimated are independent of the exact form of the assumed virus–lymphocyte and virus–virus interactions.

It is perhaps for this reason that quantification of lymphocyte dynamics can be considered to be the major contribution of mathematics to immunology.

3. CYTOTOXIC T LYMPHOCYTE RESPONSE TO VIRUSES

CTLs are a key component of our acquired immune response to viruses. CTLs are able to detect virus-infected cells by recognizing viral peptides presented in association with major histocompatibility complex (MHC) class I molecules on the surfaces of infected cells. CTLs depend on cell lysis and the secretion of inflammatory cytokines to limit virus replication. There is therefore a trade-off in all virus infections between the good effects—virus clearance—and the harmful effects—tissue damage—exerted by CTLs. The balance of good and harm done by antiviral CTLs depends on two main factors: the cell types or organs that are infected and the kinetics of the response. A limited quantity of short-term damage to a non-critical tissue is a price that is often worth paying for clearance of a virus. However, in a chronic response, the harm caused by persistent tissue destruction might outweigh any beneficial effects of reducing the viral spread.

Figure 1. Feedback loop between virus-infected cells and virus-specific CTLs. (a) The presence of a large number of virus-infected cells stimulates a large cellular immune response with many virus-specific CTLs produced. (b) The large number of virus-specific CTLs lyse the infected cells, substantially reducing the number of virus-infected cells. + indicates the death of virus-infected cells. (c) The reduced number of virus-infected cells results in reduced production of virus-specific CTLs. (d) Owing to the reduced number of CTLs virus-infected cells are able to propagate unchecked and the number of virus-infected cells increases.

It is a matter of common observation that certain viruses cause severe disease in some hosts, but inapparent or trivial disease in others. Therefore, two central questions that arise regarding the CTL response to the virus are as follows.

- (i) Is the net effect of the specific CTL response to the virus beneficial or harmful?
- (ii) Do differences between individuals in the CTL response account for differences in the outcome of the virus infection?

To answer these questions, it is necessary to quantify the specific CTL response to a virus. How should this be done? The natural approach is to use the number or frequency of virus-specific CTLs as a measure of the strength of the response. It is reasonable to assume that, at any instant, the force of an antiviral CTL response is proportional to the number of such cells. But two important complications arise. First, specific CTLs proliferate in response to the viral antigens, and therefore the CTL frequency will depend on the abundance of antigen. However, the specific CTLs reduce the abundance of viral antigen by killing virus-infected cells. This negative feedback loop (figure 1) precludes simple inferences from

measurements of specific CTL frequency. Second, an equilibrium may be established between a persistently replicating virus and the host immune response; the viral load present at equilibrium in one infected host can differ by more than 10-fold from that in another host, despite a similar frequency of virus-specific CTLs in the two hosts.

These considerations led to the hypothesis that individuals differ in the efficiency of their CTL response to a virus. In this hypothesis, as elsewhere in biology, the use of mathematics makes three important contributions:

- (i) to clarify our thinking on the factors that are sufficient to explain the observations;
- (ii) to define these factors in precise terms; and
- (iii) to suggest experiments to quantify the factors.

We illustrate these principles here by reference to two examples of persistent viral infection: HTLV-I and HIV-1.

(a) *Cytotoxic T lymphocyte efficiency*

HTLV-I infection is typically accompanied by a strong CD8⁺ T-cell response to the virus (Bangham 2000). The HTLV-I-specific CD8⁺ T cells are abundant and chronically activated, and most of these cells appear to recognize a single HTLV-I antigen—Tax, the transactivator of viral

transcription (Daenke *et al.* 1996). The frequency of CD8⁺ T cells that recognize a dominant HLA-A2-restricted epitope in Tax (Tax 11–19) differed little between asymptomatic HTLV-I carriers (median frequency of 1% of circulating CD8⁺ T cells) and patients with the HTLV-I-associated inflammatory disease known as HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP) (median frequency of 2%; Jeffery *et al.* 1999). However, the median proviral load in the HAM/TSP patients is 16-fold greater than in asymptomatic carriers (Nagai *et al.* 1998). Furthermore, the *tax* gene of HTLV-I is subject to positive selection in asymptomatic HTLV-I carriers, but not in HAM/TSP patients (Niewiesk *et al.* 1994), and we have suggested that the likely selection force is the strong anti-Tax CTL response.

Based on observations in HTLV-I and HIV-1 infection, Nowak & Bangham (1996) developed a dynamic model of the interplay between a persistent virus and the host response. It was assumed that individuals differ in the efficiency of their virus-specific CTL response. Here, the concept of CTL ‘efficiency’ or ‘responsiveness’ was embodied in the equations in two parameters: *c*, the rate of proliferation of virus-specific CTLs in response to a given concentration of antigen, and *p*, the rate at which a single virus-specific CTL kills a virus-infected cell.

The model demonstrated that it is possible for individuals to have widely differing equilibrium proviral loads despite similar (or equal) equilibrium frequencies of virus-specific CTLs. This difference in equilibrium proviral load is caused by differences in the efficiency of the CTL response to the virus (i.e. in the parameters *c* and *p*).

(b) *Correlation between CD8⁺ T-cell frequency and viral load*

Ogg *et al.* (1998) give evidence of a significant negative correlation between the plasma load of HIV-1 and the frequency of HIV-1-specific CTLs. This observation was widely interpreted as evidence that a strong CTL response to HIV-1, i.e. in an individual with a high frequency of HIV-1-specific CTLs, is associated with successful control of HIV-1 replication. However, the complicated feedback loop between CTL frequency and viral load discussed earlier (figure 1) confounds such a straightforward interpretation. It can be shown that simple models of viral–CTL dynamics predict positive, negative or zero correlations depending on the exact form of the model. This analysis demonstrates that the supposition that an efficient CTL response would result in a negative correlation between viral load and CTL frequency is based on a number of untested implicit assumptions. In this case, mathematics is limited to qualifying the initial interpretation: without more detailed knowledge of virus–lymphocyte interactions it is not possible to predict with any certainty the expected correlation between viral load and CTL frequency.

(c) *The balance of good and harm done by virus-specific CTLs*

Virus-specific CTLs can be solely responsible for clearing a viral infection from an individual. However, the same cell population in another host can cause serious, even fatal, disease. A clear example of this is seen in lymphocytic choriomeningitis virus (LCMV) infection, in which

CD8⁺ cells are the main factor responsible for clearing the infection, but can also cause fatal choriomeningitis (Buchmeier *et al.* 1980). What determines the balance between the beneficial and the harmful effects of anti-viral CTLs?

This question is sharpened by the following point: the virus-specific CD8⁺ T cells that respond most efficiently to a virus (i.e. proliferate quickly and kill quickly) should also be those that most readily produce interferon- γ (IFN- γ), tumour necrosis factor- α (TNF- α) and other pro-inflammatory substances. Therefore the CD8⁺ T cells that are most beneficial, in terms of rapid viral clearance, should also be the most harmful, in terms of inflammation and tissue damage.

There is a lively ongoing debate about the balance of good and harm done by virus-specific CTLs in HTLV-I infection, with some evidence for both effects. An observation by Valitutti *et al.* (1996) that CD8⁺ T cells produce different types of response at different densities of the MHC–peptide ligand forms the basis of a theoretical model explaining the apparently contradictory data in HTLV-I infection. Asquith & Bangham (2000) showed that individuals with a high responsiveness to a virus (high *c* and high *p* values) could suppress the equilibrium antigen concentration below the threshold required to stimulate cytokine secretion, whereas a ‘poor’ T-cell responder could not (figure 2). Thus, in a strong CTL responder the net effect of the CD8⁺ T-cell response is beneficial, while in a weak CTL responder the same cell population can contribute to inflammation and tissue damage. This theoretical model was later supported by new evidence (Vine *et al.* 2002) (figure 2*b*), which suggested that TNF- α exerts a harmful effect in HTLV-I infection only in individuals whose proviral load exceeds a threshold value.

(d) *What is the effect of CD8⁺ T-cell fratricide on the immune control of a viral infection?*

Recently, an unexpected observation was made in HTLV-I infection: HTLV-I actively infects not only CD4⁺ T cells *in vivo*, but also CD8⁺ T cells—including HTLV-I-specific CD8⁺ T cells. Furthermore, the infected CD8⁺ T cells become susceptible to lysis by autologous Tax-specific CTLs, a phenomenon called T-cell fratricide (Hanon *et al.* 2000). What is the likely effect of such CD8⁺ T-cell fratricide on the viral load and the consequent risk of disease? At first glance it appears obvious that fratricide will always increase the viral load. However, the situation is complicated because the expression of HTLV-I proteins drives the proliferation of infected cells and could thereby cause a net increase in the number of virus-specific CD8⁺ T cells. A theoretical model was developed to investigate fratricide (Asquith & Bangham 2003) (Appendix A). It was shown that fratricide could cause an increase or a decrease in equilibrium viral load and conditions were derived to determine which was the case for a given individual. In five patients studied it was shown that fratricide had probably caused a small increase in viral load. It was also shown that fratricide will always reduce the probability of viral clearance and that clinically this is likely to be the more significant effect of fratricide. The model dependency of these results was reduced in two ways. First, a general model with a minimal number of assumptions was studied analytically (so that the results

Figure 2. (a) In a theoretical model (Asquith & Bangham 2000) it was predicted that infected subjects with an efficient CTL response (rapid rates of CTL proliferation, lysis and cytokine production) should have a low proviral load and a low risk of HAM/TSP (correlated with the production of inflammatory cytokines such as TNF- α and IFN- γ). Infected subjects with an inefficient CTL response should have a high proviral load and a high risk of HAM/TSP. There is a sharp increase in the risk of disease once proviral load increases above a threshold—corresponding to a failure to control load below levels that stimulate pro-inflammatory cytokine production. (b) This theoretical model was later substantiated by experimental work (Vine *et al.* 2002) looking at the effect of a polymorphism (TNF-863A) in the TNF- α promoter in a population of 432 infected subjects. The TNF-863A allele had no effect in subjects with a proviral load below a threshold of 3%, but in subjects with a proviral load above this threshold it was associated with a significantly increased risk of disease (odds ratio of 9.7).

derived were not heavily dependent on detailed assumptions of lymphocyte and viral behaviour) and, second, the behaviours of a large number of specific models were checked numerically to ensure that the results derived were a consistent feature of a wide range of models.

To summarize, mathematical modelling has contributed to the study of the CTL response to HTLV-I by reaching the following conclusions.

- (i) If individuals differ in the efficiency of their CTL response to a persistent virus, then the frequency of virus-specific CTLs at equilibrium is not a useful index of the effectiveness of the T-cell response. The (reciprocal of) viral load is, however, a good measure of the effectiveness of the immune response.
- (ii) In an individual with an inefficient CTL response to a persistent virus, the high antigen concentration present at equilibrium can drive the normally beneficial CTLs to secrete IFN- γ and TNF- α , so that the CTLs' net effect becomes harmful.

- (iii) Fratricide among virus-specific virus-infected CTLs will lead to an increase in the viral load, despite antigen-driven proliferation of the infected CTLs.

Furthermore, mathematical modelling has demonstrated that, even if the ratio of the per-cell rates of mitotic spread and infectious spread remains unchanged throughout the course of infection, the relative contributions of infectious spread and mitotic spread will change significantly. In the initial stages of infection, most new HTLV-I-infected cells are produced by cell–cell spread of the virus, generating the observed pattern of polyclonal infection of the T cells in the newly infected host, albeit with proliferation of certain clones. But, in the later stages of infection, when equilibrium has been reached between the immune response and the replicating virus, mitotic proliferation of provirus-positive cells is the primary force maintaining the proviral load (Wodarz *et al.* 1999; Wodarz & Bangham 2000).

We conclude that it is possible to reconcile the evidence for persistent HTLV-I replication with the restricted sequence variability of the virus (Wodarz & Bangham 2000; Overbaugh & Bangham 2001).

4. HOW ROBUST ARE MODELS IN MATHEMATICAL BIOLOGY?

Theoretical results obtained in mathematical biology are usually heavily dependent on the form of the terms in the model used. For instance, a term describing the rate of infection of uninfected lymphocytes by a virus should arguably be an increasing function of viral load. However, the exact form that this increasing function should take is not clear. Should it show a linear increase with viral load (the simplest minimal choice)? Should it increase as a square or other positive power (because, for example, activated lymphocytes are easier to infect)? Or, should it saturate at some point (as infection takes a finite time and/or only a subset of putative target cells may actually be susceptible)? Such details are not known, yet they could have a profound effect on the predictions made by mathematical models. This lack of detailed knowledge—which extends to virtually every interaction routinely modelled—undermines much theoretical biology because it is difficult to build with confidence on results that are known to be model dependent.

There are at least two ways of attempting to tackle this problem. The first is to improve our detailed knowledge of lymphocyte–lymphocyte and virus–lymphocyte interactions so that we can construct the ‘correct’ model. The second is to build models that are less dependent on the exact details of the interactions. We briefly discuss these two options in §§ 4a and 4b.

(a) Constructing the ‘correct’ model

In many ways this is the ideal option. A complete knowledge of all the details of lymphocyte and viral interactions would enable the construction of the ‘one correct’ model. Such a model would have tremendous predictive power. However, it is debatable whether such complete knowledge could ever be obtained or even whether a single correct model exists. Fine details of lymphocyte behaviour are likely to vary between individuals owing to differences in genetic makeup, previous exposure to pathogens, age,

nutritional status, etc. For the modelling of all but the simplest *in vitro* systems this seems to put the construction of a correct model out of reach.

(b) *Constructing more general models*

This is a more pragmatic solution to the problem. Models can be constructed that contain general functions to describe interactions of which the details are unknown. In this way predictions can be made that will hold for all models of the form of the general model rather than just for a single *ad hoc* choice of model. The main limitation of this method is that there is invariably a trade-off between the generality of the model and its predictive power. The more general a model is, the less likely it is to be able to make a wide range of predictions; however, our confidence in the predictions that can be made will be increased. The use of this more general method of modelling in theoretical biology is illustrated by models of blood glucose control (Saunders *et al.* 1998) and by models of viral-lymphocyte dynamics (Asquith & Bangham 2003) (Appendix A).

5. CONCLUSION

The dynamics of the adaptive immune system and its response to viral infection are complex, and mathematical modelling can be a useful, sometimes vital, tool in their understanding. We have illustrated here some of the areas where mathematics has been usefully applied to immunology. The contribution of mathematics to immunology is frequently weakened by the heavy dependence of even qualitative results on the assumptions made in the model. However, mathematics has made an important contribution to understanding lymphocyte turnover, where robust conclusions can be drawn that are less dependent on model assumptions.

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APPENDIX A: GENERAL MODEL OF FRATRICIDE DYNAMICS

The model used to help understand fratricide dynamics (Asquith & Bangham 2003) is a generalized version of the basic model introduced by Nowak & Bangham (1996).

The form of the model is as follows:

$$\begin{aligned}\dot{y} &= f_1(y, z_1, z_2), \\ \dot{z}_1 &= cz_1(y + z_2) - bz_1 - f_2(y, z_1, z_2), \\ \dot{z}_2 &= cz_2(y + z_2) - bz_2 + f_2(y, z_1, z_2) + z_2 f_3(y, z_1, z_2),\end{aligned}$$

where y is the frequency of infected CD4⁺ cells, z_1 is the frequency of uninfected HTLV-I-specific CD8⁺ T cells and z_2 is the frequency of infected HTLV-I-specific CD8⁺ T cells; c is the rate of antigen-driven proliferation of *uninfected* HTLV-I-specific CD8⁺ cells and b is the rate of natural death of *uninfected* HTLV-I-specific CD8⁺ cells; and f_1 , f_2 and f_3 are completely general functions— f_1 describes the rate of change of the frequency of infected CD4⁺ cells, f_2 the rate of infection of HTLV-I-specific CD8⁺ cells and f_3 the additional net rate of growth of infected HTLV-I-specific CD8⁺ cells owing to infection.

Note that in this model infected CD8⁺ cells are not assumed to proliferate in response to antigen or to die naturally at the same rate as uninfected CD8⁺ cells. Any differences (whether positive or negative) in these terms are incorporated into f_3 . For example, if infection of CD8⁺ cells causes them to proliferate at a reduced rate c' ($c' < c$) then

$$\begin{aligned}\dot{z}_2 &= c'z_2(y + z_2) - bz_2 + f_2(y, z_1, z_2) + z_2 f_3(y, z_1, z_2) \\ &= cz_2(y + z_2) - bz_2 + f_2(y, z_1, z_2) + z_2 f_3(y, z_1, z_2),\end{aligned}$$

where

$$f_3 = (c' - c)(y + z_2) + f'_3(y, z_1, z_2).$$

Therefore the model makes no assumptions about the impact of infection on CD8⁺ cell function, the dynamics of infected CD4⁺ cells or the way in which CD8⁺ cells become infected.

The results were proved analytically using this general model. The robustness of the results to changes in the model was also checked by considering the behaviour of 128 different models numerically (including many with different forms of the CD8⁺ antigen-driven proliferation and natural death terms).

Methods of this kind help to reduce the problem of model-specific results (although, of course, the results are still far from being model independent).

It is striking that, in this case, despite our ignorance of a number of critical processes (in particular the effect of infection on CD8⁺ cell function), it is still possible to investigate aspects of fratricide dynamics.

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