REVIEWS

THE ROLE OF MODELS IN UNDERSTANDING CD8+ T-CELL MEMORY

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Abstract | Immunological memory — the ability to 'remember' previously encountered pathogens and respond faster on re-exposure — is a central feature of the immune response of vertebrates. We outline how mathematical models have contributed to our understanding of CD8+ T-cell memory. Together with experimental data, models have helped to quantitatively describe and to further our understanding of both the generation of memory after infection with a pathogen and the maintenance of this memory throughout the life of an individual.

BURNET'S THEORY OF CLONAL SELECTION
This theory states that each lymphocyte expresses antigen receptors of a single type and that antigen selects for the proliferation of clones that express receptors capable of binding the antigen.

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Immunological memory protects hosts on re-infection[1,2]. The practical importance of this phenomenon was noted by the Greek historian Thucydides, who wrote that, during an outbreak of plague in Athens, those who had recovered from the disease were best able to help the sick because they "had now no fear for themselves; for the same man was never attacked twice — never at least fatally"[3]. The most important application of immunological memory is vaccination. The discovery of vaccines based on attenuated[4] or killed[5,6] pathogens has formed the basis of the development of most of the vaccines that are currently in use[7–9]. For the most part, these developments did not require an understanding of the biological basis of immunity or immunological memory — indeed, many vaccines, including the smallpox vaccine, were in widespread use before BURNET'S THEORY OF CLONAL SELECTION[10]. These largely empirical methods for the generation of vaccines work best for pathogens that naturally cause acute infections, because recovery from acute infections is typically characterized by clearance of the pathogen and generation of long-lasting immunity. The generation of vaccines against persistent infections that do not elicit natural long-term immunity, such as infection with malaria-causing *Plasmodium* spp. or HIV, has proven to be problematic and might require advances in our current understanding of virology and immunology[11–13].

In this review, we describe some of the contributions that mathematical models have made to our understanding of various aspects of immunological memory, focusing on CD8+ T-cell responses to intracellular pathogens after acute infections. CD8+ T-cell memory to a specific pathogen (usually a virus or intracellular bacterium) can be divided into phases with different time-scales (FIG. 1). The first phase involves the primary response after exposure to the pathogen and the generation of CD8+ memory T cells specific for the pathogen. This phase is relatively rapid, occurring for a time-scale of weeks. The second phase involves the maintenance of this population of pathogen-specific 'memory' cells for a long time-scale (many years) in the absence of re-exposure to the pathogen. The third phase involves an increase in the number of memory cells after re-exposure to the pathogen, which then provides protection. Models have been developed to describe each of these phases.

Mathematical models have an essential role in understanding the complex non-linear interactions that characterize biological systems[14–16], and some of the uses and abuses of models have previously been discussed[14–16]. The development of a mathematical model typically begins with choosing a few assumptions. Analysis of the model allows us to rigorously understand the consequences of these assumptions. By adding and subtracting different processes from the model, we can determine which processes are the most important for describing different experimental observations. Models

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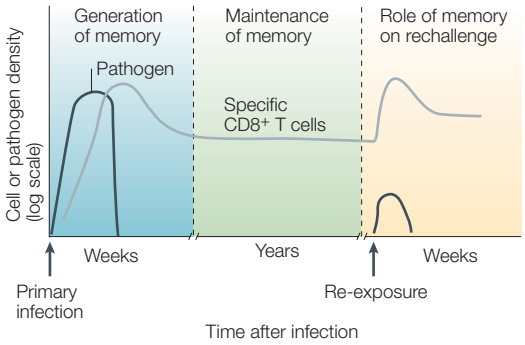

Figure 1 | **Immunological memory can be divided into distinct phases with different time-scales.** Primary infection with a pathogen results in the clonal expansion and contraction of pathogen-specific CD8+ T cells and the generation of memory cells within a few weeks. This memory-cell population can be maintained at increased levels for many years in the absence of re-exposure to the pathogen, and these cells can help control the pathogen on re-exposure.

are of most use when they make predictions that can be experimentally tested. Comparing these predictions with experimental data can lead to the rejection of the model or the gradual development of more realistic and complex models. Progress is often made when it is possible to confront multiple hypotheses (models) with data, allowing us to discriminate between different models.

Here, we describe how models have contributed to our understanding of the dynamics of the primary CD8+ T-cell response and the generation of memory cells. We then describe how models have been used to examine the longevity of CD8+ T-cell memory. Finally, we consider the role of increased numbers of pathogen-specific CD8+ T cells in providing protection after re-exposure to the pathogen.

Generation of immunological memory

The primary CD8+ T-cell response to an acute viral infection occurs for a time-scale of a few weeks. It is characterized by the rapid clonal expansion (by ~4–5 log) of virus-specific cells, generating a population of effector cells. After clearance of the infection, there is a clonal contraction (by ~1–2 log) of this population, leaving a smaller population of virus-specific memory cells[17,18]. Models have helped us to understand several aspects of this phase of the immune response.

Quantitative description of the primary response. Mathematical models have been used to estimate the precursor frequencies of epitope-specific cells (before their clonal expansion)[19] and the rate constants that characterize the clonal-expansion and clonal-contraction phases of the CD8+ T-cell response to the main epitopes of a pathogen[20,21] (in this case, lymphocytic choriomeningitis virus, LCMV). Analysis of data on the magnitude of the response to different epitopes[20,21] shows that the response can be divided into clonal-expansion and -contraction phases, during which the populations of epitope-specific CD8+ T cells change exponentially. Measurement of the parameters that describe the clonal expansion and contraction of CD8+ T cells specific for different epitopes allows us to quantitatively describe IMMUNODOMINANCE. Analysis of the data indicates that immunodominance is generated during the clonal-expansion phase rather than the clonal-contraction phase. During the clonal-expansion phase, large differences in the total number of CD8+ T cells that are specific for different epitopes can arise from small differences in either the timing of recruitment of naive CD8+ T cells to the response or the proliferation rate of activated CD8+ T cells. The next steps then include determining the cell-division and cell-death rates that underlie the net rate of change in the population of CD8+ T cells that we are describing and determining how these parameters depend on the pathogen and the genetic background of the host.

Measuring cell-division and cell-death rates. The experimental techniques and mathematical models that are required to quantitatively describe the cell-division and cell-death processes that underlie the turnover of immune cells are still being developed. The first estimates of cell-division and cell-death rates of immune cells were obtained using experimental data from the use of BROMODEOXYURIDINE (BrdU)[22] and D-glucose[23] labels, which provide us an estimate of the proportion of a cell population that has undergone division. Simple ORDINARY DIFFERENTIAL EQUATION models have been used to estimate cell-division and cell-death rates from data obtained using BrdU labelling[22,24]. However, given the limited information that can be obtained from BrdU-labelling studies, the problems that are associated with the interpretation of results[24,25] and the estimation of parameters[26] are difficult to resolve. Current approaches include the development of more informative experimental techniques together with the use of more biologically realistic models. Use of CFSE (5, 6-carboxyfluorescein diacetate succinimidyl ester) labelling allows the estimation of not only the number of cells that has divided but also the number of divisions each cell has undergone[27]. More biologically realistic models might include terms that describe the transition of quiescent cells into division[28,29] and the progression of the cell through the cell-division cycle[30]. However, the data obtained from measurements of CFSE levels might be insufficient to estimate all of the parameters of more realistic models[26]. Collaborations between experimentalists and theoreticians will be important to solve this problem.

Differentiation of CD8+ T cells into memory T cells. Mathematical models have also had a role in discriminating between different pathways for the differentiation of CD8+ T cells into memory cells during the primary response, as well as in determining the roles of antigen-dependent and antigen-independent proliferation during this phase. There are various hypotheses for the origin of memory cells during the primary response — in particular, whether memory cells are generated by the differentiation of effector cells or the converse. Experimental work has shown that cytotoxic effector

IMMUNODOMINANCE
The result of antigen(s) or epitopes within a complex mixture (such as a whole virus) being preferentially recognized or reacted against during an immune response.

BROMODEOXYURIDINE
(5-Bromo-2-deoxyuridine, BrdU). A thymidine analogue that is incorporated into DNA on replication, allowing tracking of cells that have divided.

ORDINARY DIFFERENTIAL EQUATION
A differential equation that involves ordinary derivatives of one or more dependent variables with respect to a single independent variable. For example, $dX/dt = rX$ describes the exponential growth of a population of cells, X (the dependent variable), as a function of time, t (the independent variable).

CFSE
(5,6-Carboxyfluorescein diacetate succinimidyl ester). A membrane-permeable dye that covalently attaches to free amines of cytoplasmic proteins *in vitro*. After cell division, the concentration of the label halves with each division, allowing eight to ten successive divisions to be tracked by flow cytometry.

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cells can differentiate into memory T cells[31,32], and this has been supported by the observation that a model with proliferating effector cells differentiating into memory cells can generate a good fit to the experimental data for biologically reasonable parameters[20]. A frequently used alternative model[33] that involves proliferating memory cells differentiating into non-dividing effector cells cannot generate both the clonal-expansion and -contraction phases for biologically reasonable parameters (BOX 1).

Predator–prey and programme models. The role of antigen in controlling the clonal expansion of CD8$^+$ T cells has recently been re-examined. Several experimental studies have proposed that, after stimulation, antigen-specific T cells continue to divide in a 'programmed', antigen-independent manner[34–37]. This has indicated that a revision of the earlier predator–prey-type models is required; these models assumed that the proliferation of CD8$^+$ T cells was dependent on the continual presence of specific antigen. The programmed, antigen-independent proliferation of T cells has been described using several mathematical models[38–40]. These models recapitulate the recent experimental results describing the programmed CD8$^+$ T-cell response[34–37]. Antia *et al.*[38] have used models to determine which characteristics the programme must have to be consistent with the existing data on the dynamics of CD8$^+$ T-cell responses. Their results indicate that the programme is not completely defined by the initial encounter of a T cell with antigen and might be augmented by further exposure to antigen in a brief window shortly after infection (BOX 2). They also show that programmes that reside entirely in stimulated CD8$^+$ T cells do not allow differences in the timing of recruitment of cells to the response to affect immunodominance. This indicates that the timing of the start of antigen-independent proliferation might be signalled by other cells, such as dedicated antigen-presenting cells. Programmed proliferation (unlike the earlier predator– prey models) also explains the lack of competition for antigen between the humoral and cell-mediated responses to different epitopes of a pathogen[38,41]. Using a similar modelling approach, Allan *et al.*[39] have considered how apoptosis rates can control the magnitude of a CD8$^+$ T-cell response. In addition, Chao *et al.*[40] have developed more detailed models to describe the CD8$^+$ T-cell response after immunization with killed or live pathogens.

However, although we now know that the clonal-expansion phase has both antigen-dependent and programmed components, we have yet to understand how these are delivered to T cells and have yet to describe them in a quantitative manner. In particular, we need to understand how various factors — such as 'danger' signals[42] and co-stimulatory signals that are generated by the triggering of Toll-like receptors at the surface of antigen-presenting cells[43] — regulate the antigen-dependent and -independent components of the CD8$^+$ T-cell response.

Evolutionary implications. Are there any advantages to having an immune response that commits to antigen-independent proliferation rather than constantly updating its response by tracking the pathogen? Continual updating might seem to be relatively efficient because it results in the immune response producing the necessary number of specific cells that are required to control the pathogen. An antigen-independent clonal-expansion programme is likely to be relatively inefficient — because the pathogen environment is unpredictable, the extent of clonal expansion must err on the side of caution, leading to the generation of more CD8$^+$ T cells than the minimum number that is required to clear the pathogen. However, viruses and bacteria have an extensive array of mechanisms to subvert immune responses and, potentially, any sensing apparatus[44,45]. Antigen-independent clonal expansion of CD8$^+$ T cells helps to avoid this type of subversion because the antigen-independent proliferation is set into effect when the pathogen density is low: that is, before the pathogen has the opportunity to subvert the immune response. We would expect antigen-independent proliferation to be less efficient but more robust (that is, less prone to interference from the pathogen)[38].

Longevity of immunological memory

The decline in the population number of pathogen-specific cells in the absence of re-exposure to the same pathogen is a quantitative measure of the longevity of immunological memory. The ability of pathogen-specific immunological memory to be maintained for decades in the absence of re-exposure to the pathogen was clearly documented for infection with measles by the Danish physician Panum, in 1847 (REF. 46). Quantitative measurements of changes in the levels of immune components — such as antibodies, B cells and T cells — that are associated with long-term memory have only been completed relatively recently[47,48]. In the case of CD8$^+$ T-cell memory after infection of mice with LCMV, studies have shown no detectable decline in the number of LCMV-specific CD8$^+$ memory T cells for more than 1 year after infection[18,20,49]. Studies of CD8$^+$ T-cell memory in humans have shown a slow decline in the number of CD8$^+$ memory T cells (with T-cell populations having a half-life of 8–15 years) after immunization with vaccinia virus[50–52].

Several hypotheses have been proposed to explain long-term immunological memory. In this section, we outline the main hypotheses and the contributions that mathematical models have made to our understanding of these hypotheses.

Long-lived memory cells. One possibility to explain long-term memory is that the primary response results in the generation of a population of non-dividing 'memory' cells with a long lifespan. However, this hypothesis was rejected following elegant experiments in mice that showed that memory T cells incorporate BrdU, indicating that this population is undergoing division[53–56]. These experiments showed that the turnover of naive T cells was much slower than that of memory T cells — the opposite of what might have been expected. This result also implies that the rate of

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Box 1 | Modelling the differentiation of CD8⁺ memory T cells

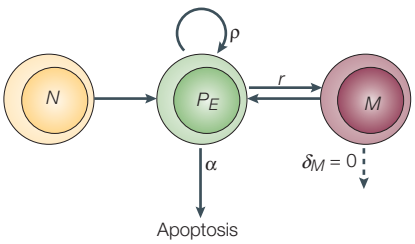
There has been considerable debate on the differentiation pathways of CD8⁺ T cells during a primary immune response and, in particular, on the origin of CD8⁺ memory T cells[17]. Here, we illustrate how mathematical models can help to discriminate between two alternative pathways for the differentiation of antigen-specific CD8⁺ T cells.

The first step is to schematically describe the two differentiation pathways. Part **a** of the figure shows the PE model, in which memory cells arise from proliferating effector cells[20]. Part **b** of the figure shows the PM model, in which proliferating cells have a memory phenotype, and these cells differentiate into effector cells[33].

The second step is to quantitatively describe these models using, for example, ordinary differential equations. In the PE model, naive cells, N, are recruited into the immune response at time (t) T_{on} after the infection and give rise to proliferating cells that have effector function, P_E. The P_E-cell population grows at the rate ρ until time T_{off}. After this, P_E cells either undergo apoptosis at the rate α or differentiate at the rate r to form memory cells, M. In the PM model, the naive-cell population, N, is recruited into the immune response at time T_{on} after infection, and these cells give rise to proliferating cells that have memory-cell properties, P_M. This cell population grows at rate ρ and differentiates into effector cells, E, at rate r until time T_{off}. After this, E cells undergo apoptosis at rate α. Because of the large amount of data that show the long-term maintenance of memory-cell populations in both models, the rate of loss of cells with the memory phenotype (δ_M) is set to zero.

The third step is to determine how well both of these models describe experimental data. We use data obtained from studies of the CD8⁺ T-cell response to the nucleoprotein 118 (NP118) epitope of lymphocytic choriomeningitis virus that occurs in BALB/c mice after infection with the virus[18], and similar results hold for other epitopes after infection of either BALB/c or C57BL/6 mice. The BEST FITS and the parameters corresponding to them are shown in the graphs beside each model. It immediately becomes clear that the PE model can fit the data with parameters that have biologically reasonable values[20] but that the PM model cannot. Essentially, when the parameters of the PM model are unconstrained, the best fit to the data is good but requires the rate constant for the clonal expansion of T cells (ρ) to be unreasonably fast, equalling 172 per day (which corresponds to cells dividing every 6 minutes, the division time being equal to ln 2/ρ). If the maximum rate of growth of T cells is constrained to a value of 5 per day (corresponding to cells dividing every 3.3 hours), then the PM model does not capture the peak and the subsequent contraction phase of the data. This observation therefore allows us to reject the PM model in favour of the PE model.

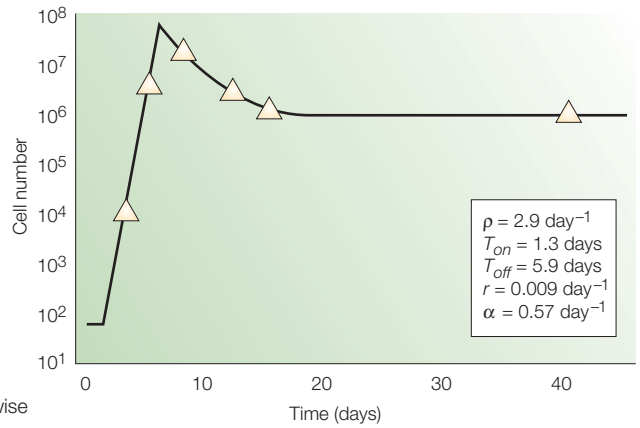
a PE model



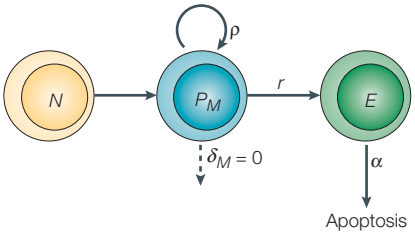
$$\frac{dP_E}{dt} = f(t)\rho P_E - [1-f(t)](\alpha + r)P_E$$

$$\frac{dM}{dt} = r[1-f(t)]P_E - \delta_M M$$

Where $f(t) = 1$, if $T_{on} \le t < T_{off}$; and $f(t) = 0$, otherwise



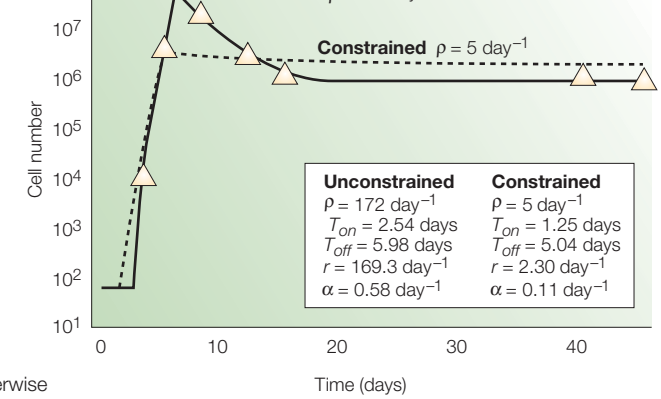
b PM model



$$\frac{dP_M}{dt} = f(t)(\rho - r)P_M - \delta_M P_M$$

$$\frac{dE}{dt} = f(t)rP_M - \alpha[1 - f(t)]E$$

Where $f(t) = 1$, if $T_{on} \le t < T_{off}$; and $f(t) = 0$, otherwise



BEST FIT
A procedure that estimates the parameters in a model by minimizing the differences between the predictions of the model and experimental data.

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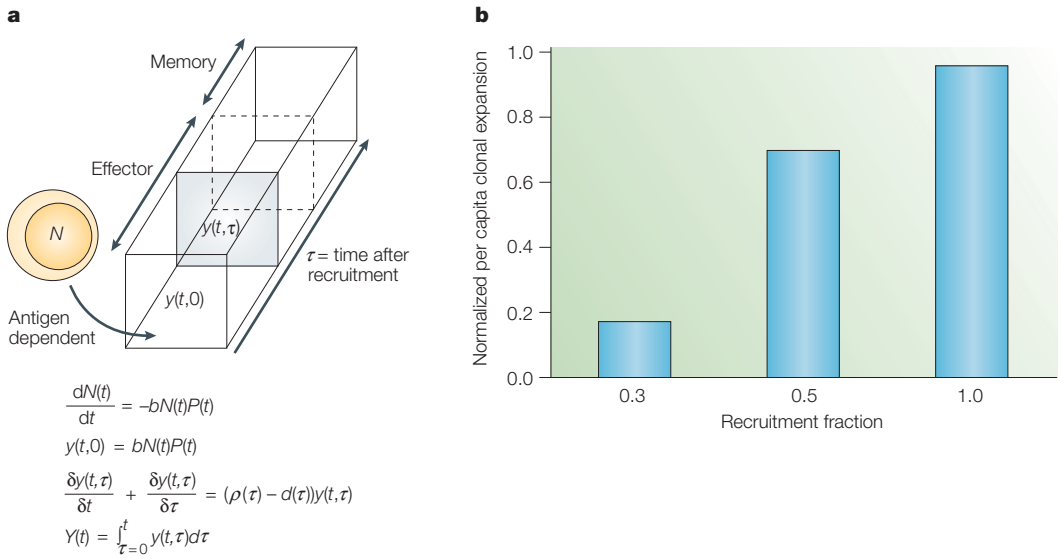
division of immune cells and the rate of death of these cells must be balanced for the population of memory T cells to be relatively stable and long-lived. Similar results have been obtained for the turnover of memory T cells in primates and humans[22,57].

Role of antigen. Given that memory cells are undergoing proliferative renewal (turnover), the central question is whether this turnover is antigen dependent or antigen independent. There has been an extensive debate on the role of antigen in the maintenance of a memory T-cell population, as well as on its role in conferring protection after re-exposure to the pathogen[17,58,59].

Experimental studies of memory initially seemed to favour the hypothesis that antigen is required for the maintenance of memory. These studies showed that the magnitude of secondary responses rapidly declined in mice that received an adoptive transfer of antigen-specific memory B or T cells in the absence of specific antigen[60,61]. However, subsequent experiments that tracked the number of antigen-specific CD8$^+$ memory T cells have led us to the opposite conclusion[62–64], and the current view is that the maintenance of populations of memory cells does not require persistent antigen. Particularly compelling are experiments that involve the transfer of CD8$^+$ T cells at least 90 days after infection from LCMV-immunized mice to non-immunized

Box 2 | **Modelling programmed CD8$^+$ T-cell responses**

The observation that, after brief stimulation with antigen, CD8$^+$ T cells continue to proliferate for many divisions in the absence of further antigenic stimulus[34,35,37] forces us to revise the way in which we model primary CD8$^+$ T-cell responses[38,39]. We describe one way in which a strictly programmed antigen-independent T-cell proliferative response can be modelled (see figure, part a). We let $N(t)$ equal the number of naive CD8$^+$ T cells at time t. After infection, these cells are recruited into the response at a rate that depends on the amount of antigen at time t, $P(t)$ and the rate constant b. The subsequent antigen-independent proliferation, death and differentiation from effector to memory cells is a function of time τ after recruitment. In particular, $y(t,\tau)$ is the number of cells per day at time t that were recruited τ days earlier, and $\rho(\tau)$ and $d(\tau)$ are the cell division and apoptosis rates, respectively, of proliferating cells at time τ after recruitment. The dynamics of the total population, $Y(t)$, are obtained by integrating $y(t,\tau)$ with respect to τ. Shortly after recruitment (when τ is small), the cells are effector cells, and later (when τ is large), they differentiate into memory cells. This model can capture the basic features of the clonal-expansion and -contraction phases of the CD8$^+$ T-cell response and the generation of immune memory[38]. The model highlights what in retrospect is an intuitive prediction: namely, that the per capita clonal expansion of CD8$^+$ T cells (that is, the clonal expansion per cell recruited into the response) is independent of the number of cells that is recruited, and consequently, the magnitude of the response is proportional to the number of cells that is recruited. If, by contrast, the programme is not strict and the clonal expansion of recruited cells can be augmented by the magnitude and duration of antigenic stimulation, then we would predict that more prolonged and larger stimulation would result not only in a greater fraction of cells being recruited to the response but also in a larger per capita clonal expansion of these recruited cells. Kaech and Ahmed[35] have shown that when the magnitude of the infection increases, the fraction of CD8$^+$ T cells that is recruited increases, as does the total response. A plot of the per capita clonal expansion versus recruitment (see figure, part b) indicates that infections with higher doses of pathogen (which also take longer to be cleared) result in greater recruitment of CD8$^+$ T cells and also greater proliferation on a per cell basis. This indicates that there are both antigen-dependent and -independent components to the clonal expansion of CD8$^+$ T cells. Understanding the relative roles of antigen-dependent and -independent proliferation during such responses is an important area for further study[117]. Part a of the figure is modified with permission from REF. 38 © (2003) Elsevier.



$$\frac{dN(t)}{dt} = -bN(t)P(t)$$

$$y(t,0) = bN(t)P(t)$$

$$\frac{\delta y(t,\tau)}{\delta t} + \frac{\delta y(t,\tau)}{\delta \tau} = (\rho(\tau) - d(\tau))y(t,\tau)$$

$$Y(t) = \int_{\tau=0}^{t} y(t,\tau)d\tau$$

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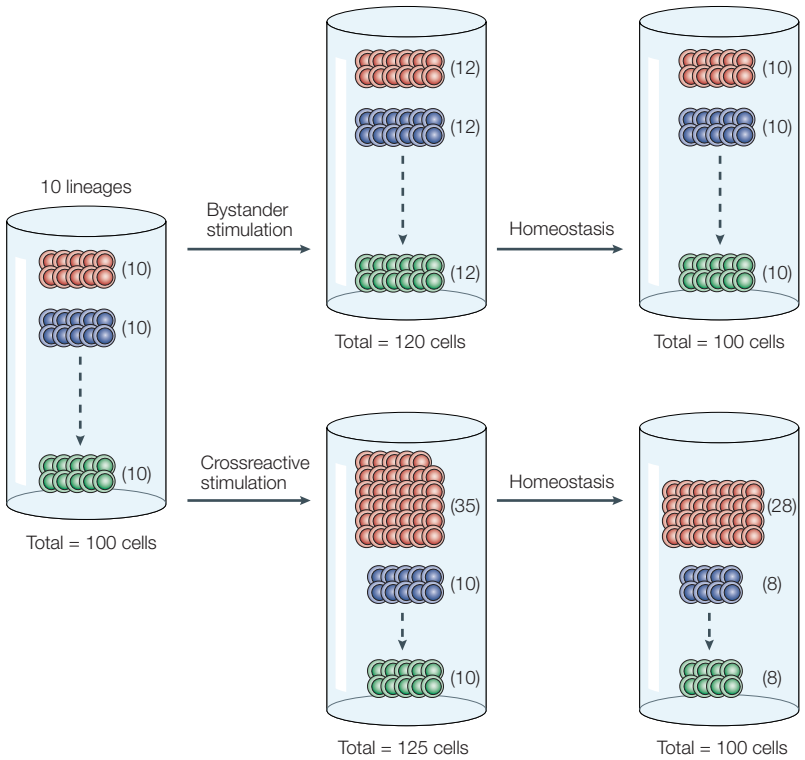

Figure 2 | **The consequences of bystander and crossreactive stimulation for the number of memory cells of different lineages in the absence and presence of homeostasis.** Here, we use a simple example to illustrate the effect of either bystander or crossreactive stimulation, together with homeostasis, on populations of different lineages of memory cells. Before stimulation, there are 10 CD8+ memory T cells in each of 10 lineages (3 lineages are illustrated) — the total number of memory cells therefore equals 100. Bystander stimulation increases the total number of cells in all memory-cell lineages by an equal amount, from 10 to 12 cells in the illustration. Homeostatic regulation then results in a proportional decline in the number of cells in all lineages, which results in a return to 10 cells per lineage. Crossreactive stimulation results in the clonal expansion of only one of these lineages (red), which clonally expands from 10 to 35 cells, whereas the number of cells in other lineages is unchanged. Homeostatic regulation then results in a proportional decline in the number of cells in all lineages, resulting in a total of 100 cells; however, there are proportionally more cells of the crossreactive lineage than of the other lineages.

BYSTANDER STIMULATION
The activation and proliferation of cells after exposure to a pathogen in a manner that is independent of their antigenic specificity.

CROSSREACTIVE STIMULATION
The activation and proliferation of (antigen-specific) cells that previously clonally expanded in response to an unrelated antigen or pathogen.

HOMEOSTATIC REGULATION
The regulation of the total number of cells of a given type, such as CD8+ memory T cells.

syngeneic mice, using protocols that minimize the transfer of antigen[62]. These experiments showed that the frequency of LCMV-specific cells remains stable in the recipient mice, even in the absence of specific antigen. It should be noted that, although these and other experiments cannot formally exclude the presence of amounts of antigen that are below the threshold of detection, they show, nevertheless, that the maintenance of memory T cells is unaffected by a severe reduction in the level of antigen. This is supported by studies showing that adoptively transferred CD8+ and CD4+ memory T cells are maintained in MHC-class-I- and MHC-class-II-deficient hosts, respectively[56,65].

Antigen-independent mechanisms. What could maintain the proliferative renewal of CD8+ memory T cells in the absence of specific antigenic stimulation? Three possibilities have been proposed: BYSTANDER STIMULATION, CROSSREACTIVE STIMULATION and HOMEOSTATIC REGULATION of turnover (FIG. 2). The bystander-stimulation hypothesis is based on the observation that infections result in the clonal expansion not only of cells that are specific for the antigens expressed by the pathogen but also of bystander cells with other specificities[54]. This bystander stimulation could be responsible for the clonal expansion of populations of memory cells that would otherwise be declining in number[66]. The crossreactive-stimulation hypothesis is based on the observation that memory T cells might have lower thresholds for stimulation than naive T cells and could therefore be stimulated in a crossreactive manner by self-antigens or after infection with unrelated pathogens[67]. Crossreactive stimulation has been observed experimentally for CD8+ memory T cells. CD8+ memory T cells that are specific for some epitopes of LCMV are stimulated by subsequent infections with unrelated viruses, such as Pichinde virus and vaccinia virus[68]. The homeostatic-regulation hypothesis is based on the observation that the total number of memory cells returns to its original level after perturbation of the size of the memory T-cell population[69], and this restricts the decline of memory-cell populations[70]. Recent studies have indicated an important role for the cytokine interleukin-15 (IL-15), possibly augmented by IL-7, in the maintenance of CD8+ memory T cells[71–73]. These three hypotheses are not mutually exclusive: there could be contributions to proliferation from bystander and crossreactive stimulation, while the total population of cells is homeostatically regulated[69]. Mathematical models have had an important role in understanding the combined effect of these three processes on the duration of immunological memory (discussed next).

Modelling the longevity of memory. There are several ways in which mathematical models have been used to investigate the duration of T-cell memory. Some models have focused on the dynamics of a single population of antigen-specific T cells[74,75]. These models indicate that, owing to the autocrine effects of the IL-2 that is produced by these cells, there could be a stable population of memory cells that is maintained in the absence of antigen. However, these models do not incorporate either the interactions between memory cells of different antigenic specificities or the constraint that is imposed by homeostatic regulation of the total population of memory T cells.

A subsequent model of immunological memory[76] (BOX 3) explicitly incorporates homeostatic regulation of the total population of CD8+ memory T cells. This model investigates how clonal expansion of naive- and memory-cell lineages after exposure to new pathogens influences the number of memory cells that are specific for pathogens that were encountered earlier. Analysis of this model shows that there are two 'rules' that govern the changes in the number of antigen-specific memory cells (BOX 3).

First, after exposure to new pathogens, the average decline in the number of cells in existing memory-cell lineages is proportional to the number of cells of new memory specificities that are generated, and this decline is inversely proportional to the size of the memory compartment. This arises because the accommodation of new memory cells in the memory compartment requires the purging of some of the pre-existing memory cells

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Box 3 | **Modelling the duration of immunological memory**

A mathematical model[76] allows us to quantify how rapidly CD8+ T-cell memory is lost after exposure to pathogens and to determine the relative contributions of homeostasis and of crossreactive and bystander stimulation to the longevity of immunological memory (see figure).

This model describes the dynamics of populations of naive- and memory-cell lineages of CD8+ T cells with different specificities. It assumes that all memory T cells have identical properties except for their antigenic specificities (and the same holds for naive T cells). The model considers how the population of existing memory cells is altered by two factors: exposure to new pathogens, and a gradual increase in the homeostatically regulated size of the memory compartment during the lifespan of an individual. Exposure to new pathogens is modelled by incorporating the clonal expansion of naive-cell lineages to populate previously empty memory-cell lineages, as well as by including bystander and crossreactive clonal expansion of cells in existing memory-cell lineages.

We first keep the size of the homeostatically regulated total memory-cell population constant and consider the consequences of exposure to new pathogens for existing memory-cell lineages. We then consider the consequences of a change in the homeostatically regulated size of the memory compartment in the absence of exposure to new pathogens. Finally, we consider the consequences of incorporating both of these processes (concurrent changes in the total size of the memory compartment and exposure to new pathogens) on the populations of memory-cell lineages.

After exposure to a pathogen, pathogen-specific naive and memory cells are recruited into the immune response. This response clears the pathogen and results in the generation of new memory cells. The memory-cell population might also clonally expand as a result of bystander stimulation and crossreactive stimulation. After pathogen clearance, the memory compartment is subject to homeostatic regulation, and the total number of memory cells returns to the homeostatically regulated value $\hat{Y}$. It can be shown that the relative decline in the total number of memory T cells present before encounter with the new pathogen, $\Delta Y/\hat{Y}$, will be determined by M, the number of memory cells of new specificities generated after exposure to a pathogen, and the total size of the memory compartment, $\hat{Y}$:

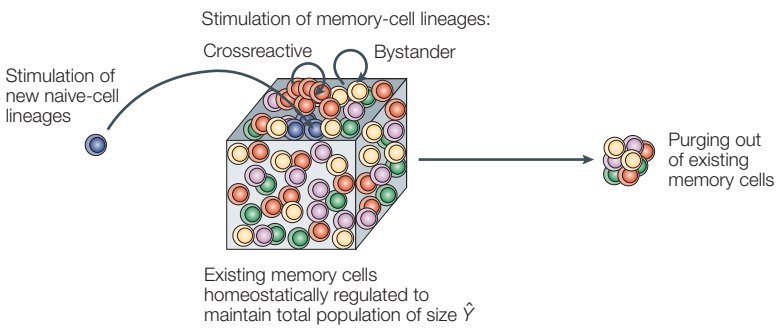
$$\frac{\Delta Y}{\hat{Y}} \approx -\frac{M}{\hat{Y}} \qquad (1)$$

Changes in the size of the total memory population in the absence of other factors will result in a proportional change in the size of all memory-cell lineages.

The combined effect of these two processes is that the average number of memory cells in lineage i at time t will be given by

$$y_i(t) = y_i(0)\exp\left[-\int_0^t \frac{m(\tau)}{\hat{Y}(\tau)}\,d\tau\right] \qquad (2)$$

where $\hat{Y}(\tau)$ is the homeostatically regulated total size of the memory population at time τ, and $m(\tau)$ is the number of new memory cells of new specificities that is generated at time τ.



(because the total size of the memory compartment is homeostatically regulated).

Second, gradual changes in the homeostatically regulated size of the memory compartment (such as the gradual increase in the size of the memory compartment with age[77]) will result in proportional changes in the average size of memory-cell lineages: for example, all else being equal, doubling the size of the memory compartment will result in doubling the average number of cells in each memory-cell lineage.

The first rule helps us to understand how bystander and crossreactive stimulation affect the longevity of memory. The magnitude of clonal expansion due to bystander stimulation should not influence the longevity of immunological memory. Intuitively, the reason for this is clear: if bystander stimulation changes (on average) the population size of all lineages by a given amount, then this change will be exactly compensated by homeostasis. In a similar manner, the crossreactive boosting of memory cells should not, on average, increase the longevity of immunological memory. This too arises because of the constraint imposed by homeostasis — the increase in the numbers of cells in the crossreactive lineages is compensated by a decline in the numbers of cells in other memory-cell lineages. However, crossreactive stimulation differs from bystander stimulation in two ways. First, crossreactive stimulation results in the clonal expansion of memory-cell lineages that are specific for the new pathogen, whereas bystander stimulation results in the clonal expansion of all memory-cell lineages, irrespective of their antigenic specificity. Consequently, although crossreactive stimulation will not result in a change in the average loss of memory, different memory-cell lineages will decline at different rates[78]. Second, crossreactive stimulation, unlike bystander stimulation, results in the clonal expansion of cells that are useful for controlling the pathogen. If there are sufficient numbers of crossreactive memory cells present, the clonal expansion of these cells can reduce the clonal expansion of naive-cell lineages, thereby increasing the longevity of memory.

The model indicates that, to estimate the average longevity of immunological memory, we need to measure both the generation of new memory-cell lineages and the size of the total memory-cell population with time. Interestingly, the longevity of memory is independent of how the total size of the memory compartment is maintained (provided that homeostasis operates in a manner independent of the antigenic specificity of CD8+ memory T cells). The model also explains the importance of separate homeostatic regulation of naive and memory compartments. This allows for the simultaneous maintenance of both a large repertoire (in the naive compartment) and long-lived populations of specific memory cells (in the memory compartment).

In future, it will be necessary to test the assumptions and predictions of this model of the longevity of memory. An important assumption that we need to verify is that all CD8+ memory T cells are identical except for their antigenic specificities. This assumption could be tested in several ways. One way would be to determine the phenotype of memory cells that are generated after

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infection with different microorganisms or immunization with different antigens[79]. Another way could involve determining whether the turnover of memory cells is independent of their antigenic specificity, how much time has passed since they were generated and how many divisions they have undergone. If there are different subpopulations or types of memory cell generated, then the rules for how these cells compete for space (homeostasis) would need to be determined.

A key prediction is that the average decline in existing memory is determined by the number of memory cells of new specificities that are generated after infection with a pathogen. Several experimental studies have considered how infection with new pathogens affects the number of $CD8^+$ memory T cells specific for previously encountered pathogens[78,80,81]. These studies used a mouse model to follow the changes in the number of $CD8^+$ memory T cells specific for different epitopes of a given pathogen (such as LCMV) after exposure to unrelated viruses (such as Pichinde virus or vaccinia virus). The studies showed that infection with a new pathogen leads to an increase in the number of cells in crossreactive memory-cell lineages but a decline in the number of cells in other memory-cell lineages. Further quantitative studies are needed to determine whether the magnitude of the changes in different memory-cell lineages is consistent with the predictions of the model or is able to reject the model. Indeed, one of the roles of mathematical models is to help indicate experiments that can reject the model.

Another area for further work is the extension of these models to consider immunological memory in humans. Measurement of the decline in $CD8^+$ memory T-cell numbers after vaccination of humans has only just begun, and the pioneering studies that measured the decline of vaccinia-virus-specific $CD4^+$ and $CD8^+$ T cells after immunization with vaccinia virus[50–52] need to be extended to consider the longevity of memory to other vaccines and to infections with pathogens. We also need to consider ways in which the longevity of memory in mice and humans might differ. One potential area in which this might occur is the loss of TELOMERES. In humans, the loss of telomeres in both naive and memory T-cell populations has been well documented[82,83], but its implications for immunological memory are less clear[84].

Finally, the rules for the regulation of the total population of memory cells (homeostasis) need to be understood on a quantitative level[85,86]. The consequences of active attrition of memory cells during the early phases of an acute response[87] also need to be incorporated into the models described here.

Memory and protection

The successful widespread use of vaccines clearly shows that immunological memory provides protection after re-exposure to antigen. It is less clear exactly how this protection is achieved. There are two possible ways: immunological memory might be able to prevent infection of the host on exposure, or it might reduce the magnitude of the infection.

Immunological memory might be able to prevent productive infection of the host after re-exposure to the pathogen in two ways. First, if the magnitude of immunological memory is sufficiently high, it can render the net growth rate of the pathogen to be negative (known as sterilizing immunity). Second, even if the magnitude of immunological memory is relatively low, it might prevent some exposures to the pathogen from resulting in productive infection of the host[88]. This happens because, initially, when the pathogen density (that is, the number of infected cells) is very low, the pathogen might become extinct as a consequence of stochastic effects. Moderate increases in the magnitude of host immunity at this time can result in an increase in the proportion of exposures that do not result in productive infection.

If immunological memory cannot prevent the initial growth of the pathogen population on re-exposure, it might nonetheless be able to control this growth faster than in a primary response (that is, reduce the peak pathogen density and the duration of infection). Several influential papers have highlighted the importance of asking (and answering) this question[33,59,89]. Factors that can result in faster control of infection after re-exposure to a pathogen include the following: an increase in the precursor frequency of pathogen-specific $CD8^+$ T cells; faster clonal expansion of memory cells (by having a shorter time-lag before the first division, a faster rate of subsequent divisions, or a lower rate of cell death); and faster or higher expression of effector functions in the responding cell population. A comparison of the clonal expansion of naive and memory populations of $CD8^+$ T cells after exposure to antigen *in vivo* indicates that memory cells have a shorter time to first division and a faster subsequent division rate[90], and that memory cells are much more efficient killers than naive cells[91]. A model of the $CD8^+$ T-cell response has indicated that an increase in the number of pathogen-specific $CD8^+$ memory T cells might not markedly reduce the peak viraemia[33]. This model is based on two assumptions: first, memory cells cannot kill (that is, do not have effector function); and second, effector cells cannot proliferate (see the PM model of differentiation in BOX 1). Recent experimental[91,92] and theoretical[20] studies indicate the need to re-evaluate both of these assumptions. Recent experiments have shown that $CD8^+$ memory T cells are much more efficient killers *in vivo* than previously appreciated[91,92]. These studies showed that memory cells can kill specific targets with kinetics similar to those of effector cells that are present at the peak of the primary response. A recent theoretical study[20] indicates a different pathway for the differentiation of $CD8^+$ T cells (BOX 1) that allows the generation of more rapid responses.

In addition to the question of how increasing the number of pathogen-specific memory cells reduces the peak pathogen density and the duration of infection, we also need to ask how these changes affect the level of pathology during the course of infection. Answering this question requires us to understand the causes of pathogenesis. So far, research into pathogenesis has

TELOMERES
Regions of highly repetitive DNA at the end of linear eukaryotic chromosomes. They protect the ends of the chromosome from shortening on replication.

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been largely qualitative: there is a vast and rapidly growing body of scientific literature about the bacterial and viral genes that are required for the virulence of pathogens[93,94]. This collection of qualitative data is a prerequisite for understanding how pathogens harm their hosts. However, it is becoming increasingly clear that understanding pathogenesis will also require understanding the dynamics of interaction between a pathogen and the immune response of the host. For example, pathology during viral infections could be caused either by the virus itself or by the killing of virus-infected cells mediated by CD8+ T cells. Mathematical models incorporating both of these processes have shown that, during persistent infections with non-cytopathic viruses, maximum pathology could arise at intermediate efficacies of the immune response[95]. When the efficacy of the immune response is very low, there is little killing either by the non-cytopathic virus or by the immune response. When the efficacy of the immune response is high, it contains the virus to very low densities, so few cells are infected and killed. When the efficacy of the immune response is intermediate, the virus infects many cells, and many of these infected cells are killed by the immune response, resulting in a high level of pathology. This and other studies considering the role of CD8+ T cells and pathogen in generating pathology are applicable to persistent infections, in which the populations of virus and virus-specific CD8+ T cells are in equilibrium. A recent study has extended this modelling framework to address the question of pathology generated by virus and CD8+ T cells during transient acute infections[96]. By varying both the number of antigen-specific T cells and the efficacy of these cells in detecting and killing virus-infected cells, this study considers the effect of immunization on pathology during the course of infection. In contrast to the model of persistent infection, this model indicates that immunological memory (that is, increasing numbers of CD8+ memory T cells) generally results in decreased pathology in response to acute infections.

We are only just beginning to understand many of the questions regarding the role of CD8+ T-cell immunity in protection. One important question within our grasp can be answered by the development of models that allow estimation of the rate at which CD8+ T cells can kill infected or target cells *in vivo*. Previous studies have focused on the measurement of killing during *in vitro* assays[97]. The development of experimental techniques that allow the measurement of killing *in vivo* has shown that *in vitro* and *in vivo* assays can yield markedly different results[91,92]. The quantitative measurement of the rate at which both effector cells and memory cells can kill infected cells will help us to understand the role of CD8+ memory T cells in controlling pathogens and reducing pathology. We are also at the early stages of understanding the dynamics of viral infections and the causes of pathogenesis[98]. The interplay between mathematical models and experimental work is likely to have an important role in the shift from our current qualitative description of virulence determinants to a quantitative description of pathology.

AFFINITY MATURATION
The increase in the average affinity of an immune response for an antigen. This occurs with time or after repeated exposure to an antigen.

Extension to CD4+ T-cell and humoral responses

In previous sections, we have discussed the generation and maintenance of CD8+ T-cell memory. Now, we briefly discuss how these considerations might be applied to CD4+ T cells and B cells.

CD4+ T-cell responses. There are many similarities between the dynamics of CD4+ and CD8+ T-cell responses, both for the generation and for the maintenance of immunological memory. After primary infection, pathogen-specific CD4+ T cells undergo clonal-expansion and -contraction phases, which culminate in the generation of CD4+ memory T cells. Similar to the CD8+ T-cell response, CD4+ T cells seem to show antigen-independent proliferation after stimulation[99]. The magnitude of the clonal expansion of CD4+ T cells is lower than that of CD8+ T cells, and estimates of the parameters that describe the dynamics of CD4+ and CD8+ T cells indicate that this occurs because of the slower clonal expansion of the CD4+ T-cell population compared with the CD8+ T-cell population[21]. A recent analysis of data on CD4+ T-cell proliferation and differentiation indicated that naive CD4+ T cells differentiate into effector cells, which in turn differentiate into memory cells[100]. Several studies have examined the generation and maintenance of CD4+ memory T cells after infection of C57BL/6 mice with LCMV[49,101,102]. These studies indicate that CD4+ T-cell memory is relatively long-lived in the absence of specific antigen but might decline faster than antigen-specific CD8+ memory T-cell populations[21]. However, studies of vaccinia-virus-specific CD4+ T cells in humans have shown that these cells persist for the same duration as vaccinia-virus-specific CD8+ T cells (that is, for decades after vaccination, with a half-life of ~10 years)[50,51]. Additional studies are needed to clarify these points.

Several studies have described the possibility that there is heterogeneity in the CD4+ and CD8+ memory T-cell populations, with different subpopulations of central memory and effector memory cells. The lineage relationships of these subpopulations and their ability to persist and confer protective immunity are not well understood[103,104].

Humoral responses. A distinguishing feature of most responses to vaccination is increased antibody levels. The dynamics of the primary humoral response to pathogens are more complex than those of the CD8+ T-cell response, requiring the coordination of both B cells and CD4+ T cells, as well as the involvement of different B-cell populations (namely, germinal-centre B cells and memory B cells, and their differentiated counterparts, plasma cells). Models can have a useful role in understanding how these populations work together[105,106], as well as in understanding the dynamics of AFFINITY MATURATION during this process[107–109].

The long-term maintenance of antibody levels after immunization has been well documented[50,51,110,111], and it does not require re-exposure to the antigen or pathogen[112,113]. The mechanisms that are responsible for the presence of antibodies many decades after the initial

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exposure are less clear. Long-term antibody production is maintained by plasma cells. These plasma cells have been shown to be long-lived cells, with an average lifespan of 3–4 months in mice[114]. So, long-lived plasma cells alone would result in a gradual decline in antibody levels with time. The slow differentiation of memory B cells into plasma cells is required to replenish the population of plasma cells[114]. One study has indicated that the differentiation of memory B cells into plasma cells depends on bystander stimulation[115], and models similar to those described for CD8+ T cells could help to understand how long-term humoral memory is affected by the extent of bystander stimulation.

Concluding remarks

In this review, we have described how mathematical models have been used to understand the generation and maintenance of immunological memory. These models have mainly focused on experimental data that were obtained from acute infections of mice with viruses that are cleared by the immune response. There are many ways in which these models can be further developed. One area for development is the extrapolation of data from mice to humans and, in particular, the consideration of how the immune system scales with changes in size (and lifespan) of the organism[116]. Another area that requires further investigation is the interaction between the dynamics of a pathogen and the immune response, particularly during persistent infections[98].

Ongoing advances in the accurate measurement of populations of immune cells, as well as the ability to use molecular techniques such as genetic knockouts to eliminate specific cell types, are resulting in the generation of data at an ever-increasing pace. Making sense of these data will require close collaboration between experimentalists and theoreticians.

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Competing interests statement

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