How lymphocytes add up

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A surprising molecular mechanism underlying signal integration and programmed proliferation in adaptive immunity has been identified. The cell cycle regulating protein Myc enables a lymphocyte to add up the strength of signals it receives and time its response accordingly.

Following a viral or bacterial infection, populations of T and B lymphocytes undergo rapid proliferation followed by a phase of substantial contraction. It is crucial that the magnitude and timing of this response is appropriate to the pathogen threat: a response that is too small or too slow could result in uncontrolled infection, a response that is too vigorous could be detrimental to the host (either because of direct immunopathology or because of the loss of the ability to respond to new pathogens). The number of divisions completed by a T lymphocyte during the first week of clonal expansion is 'programmed' soon after initial activation [1, 2, 3, 4]. Remarkably, this programmed division proceeds even if antigen is removed [1]. Multiple signals, such as antigen dose, ligation of costimulatory molecules and cytokines, synergise to determine the 'division destiny' i.e. the number of divisions that a cell undergoes after stimulation before it stops dividing[5]. Until now the molecular mechanism behind this programme was unknown. That is, how lymphocytes 'add up' the incoming signals and translate this into a regulated, appropriate number of divisions was unexplained. In the current issue of *Nature Immunology* Heinzel *et al.* identify the cell cycle regulator, Myc, as a key controller of the program of T and B lymphocyte proliferation.

The mechanism described is unexpected, but beautifully elegant. The level and timing of Myc expression following stimulation is determined by the strength and combination of signals received. Dose of cognate peptide, concentration of anti-CD28 costimulation and amount of IL-2 cytokine add linearly to determine both the post-stimulation level of Myc and the time at which the peak of Myc expression is attained in transgenic T cells (**Fig. 1**). An identical mechanism is shown to operate in naive wild type B cells in response to stimulation with the Toll-like receptor 9 ligand CpG. The lymphocytes then divide until Myc levels drop back below a critical threshold. Since, the rate at which the Myc protein declines does not depend on the initial expression, the time for Myc expression to fall below the threshold only depends on the initial stimulation received. The division destiny is therefore directly proportional to the level of Myc expression post-stimulation, and thus to the sum of the signals received.

Two models of proliferation control via Myc can be imagined. Either dilution of Myc with each cell division results in a fixed number of divisions before Myc expression drops below the threshold necessary to sustain proliferation; i.e. the mechanism is 'counter', counting the post-stimulation

divisions. Or Myc declines with time, independent of division, resulting in a fixed time before Myc expression falls below the critical threshold; i.e. the mechanism is a 'timer', timing the response post-stimulation. In the Cyton model that was previously proposed by Hodgkin and colleagues, the division destiny is determined by a counter[6]. However, in this latest work they show instead that Myc is a timer. Using a dye to track division of activated B cells, Heinzel *et al.* report that intracellular Myc expression is similar in cells having completed different number of divisions, and declines more or less exponentially over time. Heinzel *et al*. successfully validated a critical prediction of the timer hypothesis- namely that slowing the division rate (using an S-phase inhibitor) should result in fewer divisions but with cells entering quiescence at the same time.

The fact that Myc expression is not diluted by cell division suggests that Myc levels are established by a steady state of rapid production and turnover. Since the observed Myc decay rates were similar under various conditions, the dominant mechanism determining the loss of Myc should be a reduction in the production rate. By blocking protein production it was confirmed that Myc has a short (20 min) half-life, implying that the observed slow decline of Myc (half-life 7 hours) is the result of a slowly declining production rate. Thus, daughter cells have to inherit the rate at which Myc is produced, and have a similar rate at which production declines. It remains an open question how this is achieved.

Overexpression of Myc (by transduction with a Myc-expressing retrovirus in CpG activated B cells or by Myc transgene expression under the control of an enhancer/promoter vector in T cells) confirmed the causal link between Myc expression and division destiny. Cells expressing elevated amounts of Myc divided at a similar rate to control cells but remained in the proliferative program for longer, resulting in a greater number of divisions prior to quiescence. Similarly, cells sorted on the basis of their Myc expression using a fluorescent-reporter system, showed a clear relationship between Myc expression and division destiny. That Myc is the main determinant of division destiny, at least in this murine system *in vitro*, is confirmed by the very tight correlation between Myc expression after initial stimulation and division destiny: virtually all of the variation in division density is explained by variation in Myc. Finally, the observation that overexpression of Myc does not lead to uncontrolled growth indicates that cells possess an independent program determining their time to die. Manipulating the time to die, Heinzel *et al.* show that cells may undergo apoptosis during the division phase with Myc levels above the threshold, or after becoming quiescent.

Earlier work using videos [7], molecular barcoding [8], or congenic markers [9] to trace the progeny of individual cells demonstrated a large heterogeneity in the quantitative contribution of progenitor cells to the immune response. A small fraction of the progenitors undergo many cell divisions, and comprise most of the response, whereas most progenitors divide little. Fitting a novel mathematical model to their data, Heinzel *et al.* show that small changes in Myc expression, combined with small changes in the time to die can translate into large changes in the amplification of cell number. Thus, a prediction of this work is that the heterogeneity observed *in vivo* [8 9] is at least partly due to heterogeneity in Myc expression. This heterogeneity could reflect variation between progenitors in the signals received and/or variation in the translation of this signal into a level of Myc expression.

This work poses several new questions for the field. First, how is timed Myc production inherited from one generation of cells to the next? The mechanism whereby temporal changes in the rate of

Myc production are passed from mother to daughter needs to be elucidated. Second, how important is Myc-regulation for determining cell fate *in vivo*? Myc expression *in vitro* and over-expression *in vivo* [10] has profound consequences for lymphocyte proliferation. The relative importance of physiological levels of Myc relative to other cell-extrinsic and intrinsic factors during a host response is still to be determined. Additionally, do the infrequent cell divisions that lymphocytes undergo to maintain homeostasis also depend on Myc? Furthermore, what are the implications for estimates of immune cell proliferation during clonal expansion? Quantification of cell proliferation using labeling (e.g. with CFSE, BrdU or stable isotopes) is a widely used technique [11, 12, 13]. Interpretation of these experiments typically assumes that lymphocytes obey a time-independent process. Heinzel *et al* show that this assumption is inaccurate: revision of several important studies may be necessary. Finally, how does continual exposure to cytokines and growth factors extend cell division and survival? Heinzel *et al* have focussed on the first rounds of division after activation; the regulation of proliferation during an ongoing immune response remains to be studied. Is the subsequent proliferation also division-independent and does it involve continuous reprogramming of Myc levels?

In summary, this beautiful piece of work elucidates an unexpected timing mechanism underlying the lymphocyte program of division. It paves the way for a quantitative understanding of adaptive immunity.

Figure legend: Myc expression quantitatively regulates the programme of lymphocyte proliferation in response to multiple signals.

Following lymphocyte activation, intrinsic Myc expression rises to a peak that is determined by the sum of signals received. A strong signal e.g. peptide+cytokine+costimulation results in a higher and later peak of Myc (thick blue line) than a weak signal e.g. peptide alone (thin blue line). Lymphocytes divide for the period that Myc is above a critical threshold (dashed line), returning to quiescence once Myc drops below this threshold. Since Myc declines at a constant rate, the time for Myc to fall below this threshold depends on the initial signal ($t_2 > t_1$). Consequently, the length of time that a lymphocyte divides for is programmed by the integration of activating signals. In this way, a lymphocyte adds up the strength of signals it receives and responds accordingly.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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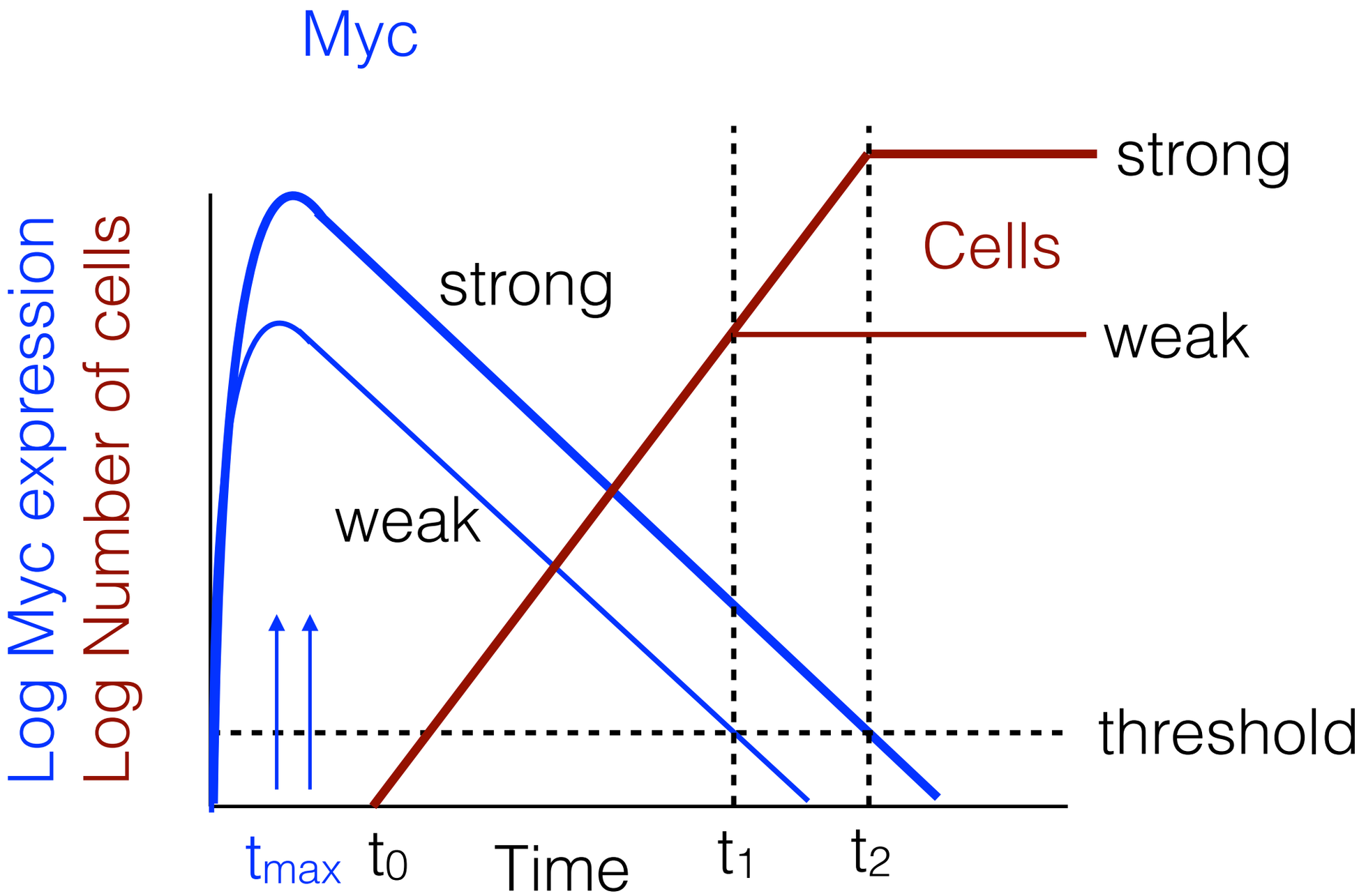
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Myc
Log Myc expression
Log Number of cells
strong
weak
Cells
strong
weak
threshold
t_{max}
t_0
Time
t_1
t_2