proportion of *i*NKT thymocytes that are committed to the adipose lineage in wild-type mice, which is technically challenging given the weak intracellular staining with the available antibodies directed against E4BP4 (as shown in this paper[3]). An E4BP4-GFP reporter mouse might be useful in addressing this point. The alternative view, that acquisition of the adipose-resident *i*NKT cell phenotype occurs in the adipose tissue in response to microenvironment-specific stimuli (**Fig. 1b**), is supported circumstantially by the known ability of adipocytes to present lipid antigens to *i*NKT cells in a CD1d-restricted fashion[11], as well as by the fact that obesity in mice can be partially reversed by the transfer of hepatic *i*NKT cells[6]. The latter result indicates that non-adipose *i*NKT cells can acquire adipose-resident anti-inflammatory functions after arriving in adipose sites, although whether transferred hepatic *i*NKT cells undergo downmodulation of PLZF expression and induction of of E4BP4 expression in adipose tissues is not addressed in that report[6]. The present study demonstrating that immature *i*NKT cells from $V_{\beta}8.2(F108Y)$ mice exhibit induction of the expression of E4BP4 and PD-1 while still in the thymus[3] appears to exclude the possibility of an absolute requirement for the adipose microenvironment in directing the development of an adipose-resident *i*NKT cell phenotype, although this could reflect the abnormal signaling capacity of the mutant TCR employed, which might somehow mimic signals that normally occur only in adipose tissues.

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How lymphocytes add up

Becca Asquith & Rob J de Boer

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

Following viral or bacterial infection, populations of T lymphocytes 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 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[1–4]. Remarkably, this programmed division proceeds even if the antigen is removed[1]. Multiple signals, such as antigen dose, ligation of costimulatory molecules and cytokines, act in synergy 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 program 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 proliferation program of T lymphocytes and B lymphocytes[6].

The mechanism described is unexpected but beautifully elegant. The amount and timing of Myc expression following stimulation is determined by the strength and combination of signals received. The dose of cognate peptide, concentration of antibody to the receptor CD28 (for costimulation) and amount of the cytokine IL-2 add linearly to determine both the abundance of Myc after stimulation and the time at which the peak of Myc expression is attained in OT-I T cells (which have transgenic expression of a T cell antigen receptor specific for an ovalbumin epitope) (**Fig. 1**). An identical mechanism is shown to operate in naive wild-type B cells in response to stimulation with the dinucleotide CpG (the ligand for Toll-like receptor 9). The lymphocytes then divide until the abundance of Myc drops back below a critical threshold. Since the rate at which the abundance of Myc protein decreases does not depend on its initial expression, the time for Myc expression to fall below the threshold depends only on the initial stimulation received. The division destiny is therefore directly proportional to the amount of Myc expression after 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 a 'counter' that counts the divisions after stimulation), or Myc expression decreases with time, independently of division, which results in a fixed time before Myc expression falls below the critical threshold (i.e., the mechanism is a 'timer' that times the response after stimulation). In the cyton model previously proposed by Hodgkin and colleagues, division destiny is determined by a counter[7]. However, this latest work[6] shows instead that Myc is a timer. Through studies using a dye to track the division of activated B cells, Heinzel *et al.* report that intracellular Myc expression is similar in cells that have completed different numbers of divisions and decreases more or less exponentially over time[6]. Heinzel *et al.* successfully confirm the following critical prediction of the timer hypothesis: that slowing the division rate (through the use of an S-phase inhibitor) should result in fewer divisions but with cells entering quiescence at the same time[6].

The finding that Myc expression is not diluted by cell division suggests that the abundance of Myc is established by a steady state of rapid production and turnover. Since the observed Myc decay rates are similar under various conditions, the dominant mechanism that determines the loss of Myc should be a reduction in the production rate. By blocking protein production, the authors confirm that

Becca Asquith is in the Department of Medicine, Imperial College London, London, UK. Rob J. de Boer is with Theoretical Biology and Bioinformatics, Utrecht University, the Netherlands.
e-mail: b.asquith@imperial.ac.uk

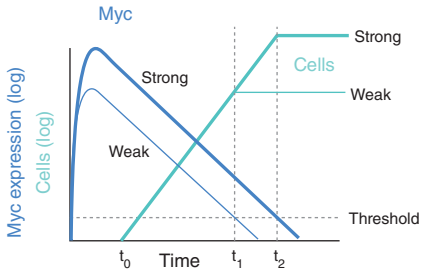

Figure 1 Myc expression quantitatively regulates the program of lymphocyte proliferation in response to multiple signals. Following lymphocyte activation, intrinsic Myc expression rises to a peak that is determined by the sum of signals received. A strong signal, such as costimulation with peptide and cytokine, results in a higher and later peak of Myc (thick blue line) than the peak that results from a weak signal, such as peptide alone (thin blue line). Lymphocytes divide for the period that Myc is above a critical threshold (dashed horizontal line) and return to quiescence once Myc drops below this threshold. Since Myc decreases 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 during which a lymphocyte divides is programmed by the integration of activating signals. In this way, a lymphocyte adds up the strength of signals it receives and responds accordingly.

Myc has a short half-life (of 20 minutes), which indicates that the observed slow decrease in Myc (a half-life of 7 hours) is the result of a slowly diminishing production rate. Thus, daughter cells must inherit the rate at which Myc is produced and have a similar rate at which production diminishes. It remains an open question how this is achieved.

Overexpression of Myc (by transduction of CpG-activated B cells with a Myc-expressing retrovirus or by expression, in T cells, of a transgene encoding Myc under the control of an enhancer-promoter vector) confirms the causal link between Myc expression and division destiny. Cells with elevated expression of Myc divide at a rate similar to that of control cells transduced with empty vector but remain in the proliferative program for longer, which results in a greater number of divisions before quiescence. Similarly, studies of cells sorted on the basis of their Myc expression (through the use of a fluorescent-reporter system) show a distinct relationship between Myc expression and division destiny. The finding that Myc is the main determinant of division destiny, at least in this mouse system *in vitro*, is confirmed by the very tight correlation between Myc expression after initial stimulation and division destiny: almost all of the variation in division density is explained by variation in the abundance of Myc protein. Finally, the observation that overexpression of Myc does not lead to uncontrolled growth indicates that cells have an independent program that determines their time to die. Manipulating the time to die, Heinzel *et al.* show that cells can undergo apoptosis during the division phase with the abundance of Myc above the threshold, or after becoming quiescent[6].

Earlier work using videos[8], molecular barcoding[9] or congenic markers[10] to trace the progeny of individual cells demonstrated considerable heterogeneity in the quantitative contribution of progenitor cells to the immune response. A small fraction of progenitor cells undergo many cell divisions and constitute most of the response, whereas most progenitor cells 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[6]. Thus, a prediction of this work is that the heterogeneity observed *in vivo*[9,10] is due at least in part to heterogeneity in Myc expression. This heterogeneity could reflect variation between progenitor cells in the signals received and/or variation in the translation of this signal into an amount 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 for the determination of cell fate *in vivo*? Myc expression *in vitro* or overexpression *in vivo*[11] has profound consequences for lymphocyte proliferation. The importance of the physiological abundance of Myc relative to that of other cell-extrinsic and intrinsic factors during a host response remains to be determined. Additionally, do the infrequent 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 by labeling (for example, with the division-tracking dye CFSE, the thymidine analog BrdU or stable isotopes) is a widely used technique[12–14]. Interpretation of such experiments typically assumes that lymphocytes obey a time-independent process. Heinzel *et al.* show that this assumption is inaccurate[6]; thus, revision of several important studies might be necessary. Finally, how does continual exposure to cytokines and growth factors extend cell division and survival? Heinzel *et al.* focus on the first rounds of division after activation[6]; the regulation of division destiny during an ongoing immune response remains to be studied. Is the subsequent division density also division independent, and does it involve continuous reprogramming of Myc abundance? In summary, this beautiful piece of work elucidates an unexpected timing mechanism that underlies the lymphocyte program of division. It paves the way for quantitative understanding of adaptive immunity.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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