Population dynamics of B cell subsets – analysis and predictions

We aimed to quantify the dynamics of various subsets within mature B cell population and to understand the rules of replacement of old cells by that of new ones within each subset. We adopted the conventional view of B cell development and assumed that Transitional 2 (T2) cells are the direct precursor of FM cells, which then participate in germinal centre (GC) reactions upon antigen interaction. We also assumed that both FM and T2 cells circulate freely in the lymphatic system, and so we pooled the numbers of these subsets in spleen and the lymph nodes when modelling their dynamics.

Follicular Mature B cells

Replacement kinetics are consistent with FM cells being a single, kinetically homogeneous population, with cell lifetime increasing with host age

The normalised donor fraction f_d in the FM compartment stabilises close to 1 by about 120d post-BMT, implying the near-complete replacement of the compartment within that timeframe. Complete replacement suggests that the average rates of loss across host and donor cell populations are always equal. We modelled this behaviour by assuming that cells differentiate into FM B cells at a rate proportional to numbers of T2 cells, and all FM cells, whether host or donor, are lost through death or differentiation at rate δ and renewed through division at rate ρ. For generality we allowed either of these rates to vary with the age of the host. We fitted each model simultaneously to the timecourses of the total size and donor cell chimerism of the FM population (Figure 1A and B; see Methods for details). We found strongest support for a model in which the loss or turnover rate δ changes with host age and the division rate ρ remains constant. Specifically, total numbers of FM B cells are given by

$$\frac{\mathrm{d}N_{\mathrm{FM}}}{\mathrm{d}t} = \phi(t) + (\rho - \delta_0 e^{-rt})N_{\mathrm{FM}}, \tag{1}$$

where $\phi(t)$ is the daily rate of influx from T2, which changes little with host age but whose timecourse was estimated using a (nearly flat) spline, and time is measured from age 40 days, at which time the loss rate is δ_0. This model was superior to the simplest model with constant rates of division and turnover (ΔAIC = 5.9) and also superior to the alternative with a time-varying division rate (ΔAIC = 12). We estimate that FM B cells divide slowly, on average every 56 days, and have a mean residence time (lifetime) of 18 days in 40 day-old mice. This life expectancy doubles approximately every 14 months. We also predict that approximately 4% of FM B cells are replaced each day by newly differentiated cells from the T2 population. Parameter estimates and 95% confidence intervals are in Table 2. We also define the net loss rate λ as the aggregate of cell division and turnover (i.e. $\delta - \rho$), which decreases with time for FM cells, as δ declines. Therefore, in old animals individual FM clones and their progeny would persist longer in follicles than in younger animals, purely due to age-mediated changes in the host environment.

No evidence for heterogeneity or cell-age dependent effects within FM B cells

The decline we detect in λ with host age, which we infer derives from progressively increased survival within the FM compartment, therefore drives a gradual slowing of the approach to stable chimerism relative to the kinetic predicted by a simple model of constant division and turnover. An alternative explanation of this time-varying kinetic is that the FM pool comprises independent sub-populations with different but constant rates of division and turnover, each fed from the T2 source. In this scenario, less persistent populations (those with a high net loss rate λ) will be replaced most rapidly after BMT, giving an initial steep upslope in chimerism. There will then follow a slower increase as the more persistent FM subpopulations (with low λ) are replaced by donor cells relatively slowly.

We fitted a model of kinetic heterogeneity assuming two independent subpopulations, allowing their relative size and their constant loss rates λ_1 and λ_2 to be free parameters. However this model received lower support than the model of FM cells as a single population with turnover slowing with host age (ΔAIC = 12, Table 1). Indeed there was a very weak signature of kinetic heterogeneity; the estimated net loss rates of the two FM subpopulations were nearly equal ($\lambda_1 = \delta_1 - \rho_1$ = 0.039 (0.025, 0.062) and λ_2 = 0.028 (0.023,0.033)) and close to that of the simplest homogeneous model with λ = 0.029 (0.024, 0.033).

We also found no evidence for any host-donor differences in kinetics in the form of a persistent host-derived 'incumbent' population, or any change in the net rate of loss of loss with cell, rather than host, age (Table 1). For a discussion of these models, see Hogan et al. PNAS 2015 and Rane et al. PLoS Biology 2018 (in press)).

Another potential mechanism for slowing replacement with host age is a decline in the rate of influx (e.g. a fall in the rate of differentiation from T2) with host age. We found no evidence for this (ΔAIC > 10), and were close to overfitting at this point.

The model of time-varying loss successfully predicts the kinetics of Ki67 expression within host and donor populations

As well as the numbers of host and donor-derived cells in the FM compartment, we also measured the kinetics of their expression of Ki67, a nuclear protein expressed during cell cycle and lost with a lifetime of 3-4 days following mitosis (Gossel eLife 2017 and others - see refs in that paper). Immediately following BMT the donor FM cells are highly enriched for recently divided cells, with around 80% Ki67^{hi}, but this proportion falls slowly to equalise with that of host cells at around 10% after approximately 100 days (Figure 1C, red and green points).

As a validation of the models, which were fitted only to the timecourses of total FM B cell numbers and chimerism, we used them to predict the dynamics of Ki67 expression within host and donor cells over time. The best-fitting time-dependent homogeneous model predicted these kinetics remarkably well. These predictions were generated by inserting the estimated rates of loss ($\delta(t) = \delta_0 e^{-rt}$) and division ($\rho$) into a model which explicitly follows the transit of cells between Ki67^{hi} and Ki67^{lo} states, which we have employed previously (Hogan PNAS 2015, Gossel eLife 2017). We assumed a mean lifetime of Ki67 post-mitosis of 3.5d, and also assumed that Ki67^{hi} and Ki67^{lo} cells are lost at equal rates $\delta(t)$. The timecourses of host and donor Ki67^{hi} fractions were then generated by simulating the model with its best-fit parameters, beginning at the mean Ki67^{hi} fractions observed at 12d post-BMT, pooled across all experimental cohorts. See Methods below for details.

The simulations show that the host/donor disparity in Ki67 expression derives largely from residual expres-

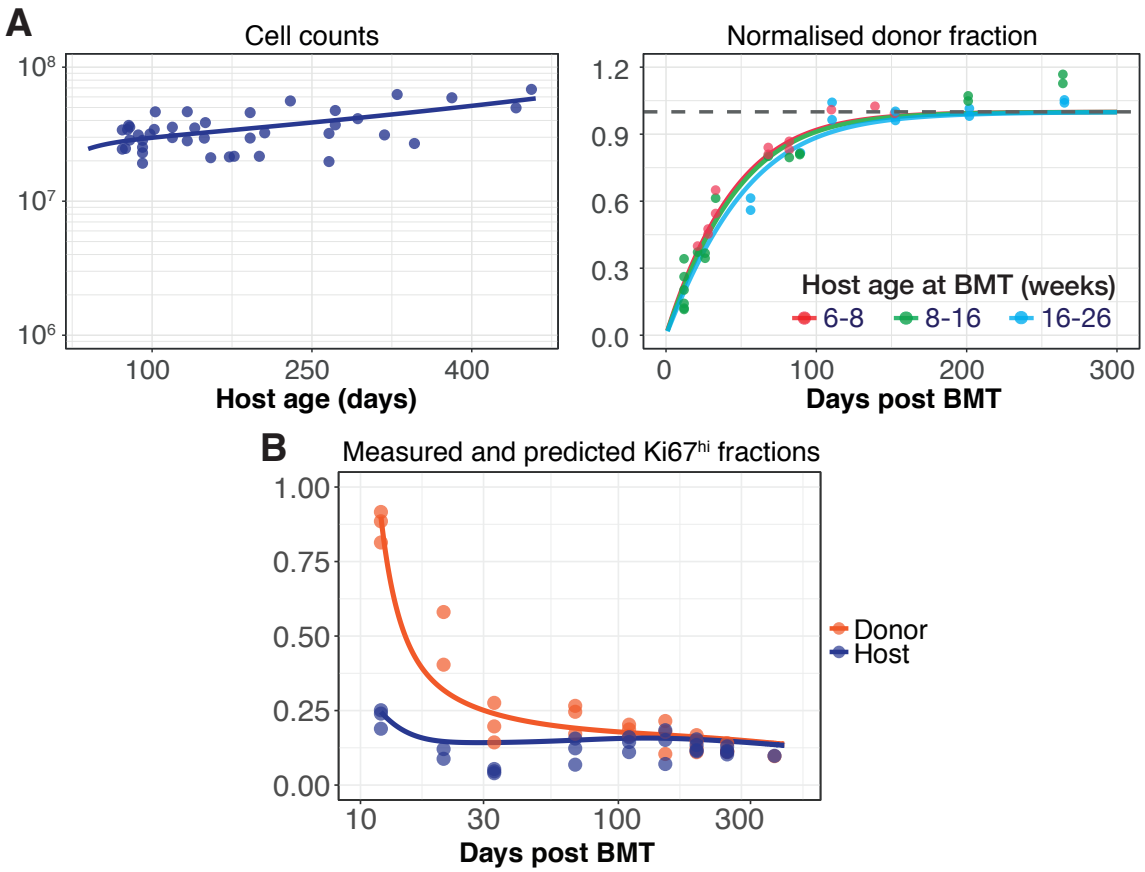

Figure 1: **Fitted and predicted population dynamics of FM B cells, using the best-fitting model in which cells divide at a constant rate and their mean residence time increases with host age.** (A) The model was fitted simultaneously to the extended timecourses of total cell counts of FM B cells pooled from LN and spleen in busulfan chimeras, and the donor fractions in FM B cells normalised to the chimerism in T2 cells. The latter took account of different ages at BMT (right-hand panel, coloured lines; each generated using the mode of the age within each group). (C) We then used the model parameters to predict the proportion of cells that were Ki67^{hi} within host and donor FM B cells over time.

sion of Ki67 on cells that have recently entered the FM pool from the T2 precursor population T2s are not dividing, you say... so surely this is further evidence that T1s are the source for FMs?. Soon after BMT, the donor FM pool is highly enriched for these recent immigrants, roughly 80% of which are Ki67, relative to the more established host-derived pool. The lifetime of FM cells is relatively short and so a substantial fraction of newly immigrated Ki67^{hi} cells are lost before they transition to Ki67^{lo}. This interplay means that quiescent donor cells are slow to accumulate. In parallel, Ki67 levels transiently dip in host FM cells following BMT due to the sudden reduction in influx from the host T2 pool, before they re-establish equilibrium at lower numbers.

Summary

- Most support for model in which FM B cell residence time increases with host age
- No evidence for kinetic heterogeneity (*i.e.* multiple subpopulations with different turnover, or host

incumbents)

- No evidence for changes in the per-cell rate of differentiation from T2 with cell age
- No evidence for changes in rates of loss or division of FM cells with cell age.

Model and ΔAIC				
Time-dependent	Simple homogeneous	Kinetic heterogeneity	Incumbent	Age-structured
0	5.9	12	12	12

Table 1: **Comparison of models describing population dynamics of Follicular Mature (FM) B cells, pooled from LN and spleen**. The models assume that FM B cells derive directly from Transitional 2 B cells. AIC values are shown relative to that of the best fitting model, in which the rate of loss (turnover) of FM cells declines slowly with the age of the host. Predictions of more complex models were very close to those of the simple homogenous model (that is, either very little kinetic heterogeneity, close to zero incumbent cells, or effects of cells age on turnover or division rates)

.

Parameter	Estimate	95% CI
Total cell numbers at age 7 wks ($\times 10^{-6}$)	24	(11, 54)
Total daily of influx from T2 into FM at age 7 wks ($\times 10^{-6}$)	1.1	(0.95, 1.3)
Mean residence time (days) at age 7 wks	18	(7.5, 41)
Mean inter-division time (days)	56	(3.5, 900)
Time for mean residence time to double (days)	430	(130, 1500)

Table 2: **Parameter estimates from the best-fit (time-dependent) model for FM B cells (Spleen + LN)**. Confidence intervals were estimated using the inverse of the Hessian matrix at the ML estimates of the parameters.

Note: Age-structured model gives visually bad fits for FM cells.
When fitting FM cells with the incumbent model, size of the incumbent population is estimated to be ≈ 0, making it equivalent to constant birth-death model. When fitting MZ model the counts of incumbent cells are estimated $\approx 10^5$.

Model	Source	ΔAIC	Mean residence time (d)	Residence doubling time (d)	Mean interdivision time (d)	Mean clonal lifetime(s) (d)
Time-dependent	T1	0	18 (7, 44)	250 (46, 1300)	69 (2, 2800)	–
	T2	1.3	17 (6, 54)	520 (110, 2800)	56 (3.5, 900)	–
Age-structured	T1	27	20 (16, 26)	NA	89 (36, 210)	–
	T2	21	33 (21, 39)	NA	2000 (1300, 3300)	–
Simple	T1	26	–	–	–	40 (35, 47)
birth-death	T2	13	–	–	–	34 (30, 41)
Incumbent	T1	26	–	–	–	39 (34, 47)
	T2	13	–	–	–	34 (29, 40)
Kinetic	T1	26	–	–	–	41(37, 46)
heterogeneity						33 (10, 102)
	T2	13	–	–	–	17 (6.0, 54)
						26 (14, 47)

Table 3: **Comparison of AIC values and parameter estimates using either T1 or T2 as the source population from different models fitted to cell counts and donor fractions† in FM B cells (Spleen + LN).**

† For the incumbent and constant birth-death model we only have λ estimates.

* r is the rate of change of residence-time with host-age or cell age, hence log(2)/r denotes the average time taken for mean residence time 'τ' to double.

Changing ρ with time or cell age gives (visually) poor fits hence not included in this analysis.

NA - estimates for 'r' are close to zero therefore log(2)/r $\sim$ NA/Inf. This shows that in this case there is very little or no effect of cell age on $\delta(a)$.

Germinal Center B cells

Invasion kinetics of donor-derived GC cells differ between spleen and lymph nodes.

GC cells are assumed to derive directly from the mature follicular cells [REF] which are known to circulate freely between spleen and lymph nodes [ref]. Therefore, we use pooled numbers of spleen and LN FM cells as a common source for all GC B cell subsets. We find that in both spleen and lymph nodes, the chimerism in GC B cells reaches to the level of chimerism in their source (FM) population (Figure 2 B), showing complete replacement of host GC cells by that of donor-derived cells. Interestingly, the turnover of GC B cells in the spleen is more rapid than in lymph nodes, with the normalised donor fractions in spleen stabilising after approximately 120d post-BMT, while it takes twice as long ($\sim$ 250d) in the lymph nodes. Due to this disparity in the invasion kinetics of donor cells between the splenic and LN GC pools, we model them separately with an assumption that the same freely circulating pool of FM B cells feeds into both, spleen and LN GC populations with a constant rate of influx, over time.

B cells participating in GC reactions follow same kinetics of division and loss, irrespective of the age of the host.

We modelled the behaviour of GC cells using the similar strategy that was employed for FM B cells and began with the fairly general model that allowed either the rate of division ρ or turnover δ to vary with host-age. Varying the rate of cell division with time fits poorly to the time-course of cell counts and normalised donor fractions and yields un-physiological estimates for the population size at t_0 (7 weeks of mouse age) . On the contrary, the dynamics of GC B cells in both splenic and LN GC populations were described very well (at-least visually) by the model in which the propensity of GC cells to die and/or differentiate declines gradually with host-age while their ability to renew by division stays unaltered.

Interestingly, we find that δ changes extremely slowly with host-age in this model, such that it takes approximately 130 and 84 months for the mean residence time to double in splenic and LN GC populations, respectively. We speculate that the time-dependent variations in the host environment has little or no effect on the turnover of B cells that participate in GC reactions and the population dynamics of GC B cells can be explained by rates of division and turnover that do not vary with host age. The simplest model with constant division and turnover provided better visual description of the time-course of cell counts and normalised donor fractions (Figure 2 A and B) and received stronger statistical support as compared to the model where δ declines with host-age (Δ AIC = 2 and 3, respectively). The total size of GC compartment is therefore given by,

$$\begin{aligned} \frac{dN}{dt} &= \phi(t) + \rho\, N - \delta\, N, \\ \frac{dN}{dt} &= \phi(t) - \lambda\, N \qquad\qquad \Rightarrow \lambda = \delta - \rho. \end{aligned} \tag{2}$$

where, λ is the net loss rate and ϕ is the influx of FM cells in the GC compartment at a given time 't'.

Consistent with the disparity in donor fraction kinetics in spleen and lymph nodes, the mean life-time of a clonal lineage (given by the inverse of λ) of LN GC B cells was estimated to be $\approx$ 3 fold longer than the spleen GC cells (15 and 41 days respectively, Table 5). We estimate that $\approx$ 2% LN GC and 6% of spleen GC population is replaced everyday by new cells slowing in form Fm compartment. Parameter estimates with 95% confidence intervals are in table 5. It can be inferred from this analysis that GC reactions have different

half-lives between different secondary lymphoid organs and their dynamics are primarily regulated by the tissue-specific factors.

GC population is maintained by continuous seeding of follicular cells into a kinetically homogeneous population over time.

Each instance of GC reaction typically lasts for about 3-4 weeks [Can kesmir and Rob de Boer JI 1999, L. Mesin et al., Immunity 2016]. Therefore, the turnover or division of GC B cells may not be influenced by the cell-intrinsic changes accumulated as a function of their time spent in germinal centres. Moreover, intermittent expansion and selection events followed by rapid kinetics of collapse of GC clusters [Nilushi De Silva and Ulf Klein, Nat. Rev. Imm. 2015, N. Wittenbrink et al., JI 2010] suggest that majority of GC cells may have lifetimes much shorter than 3 weeks. Therefore, we refrained from using complex models that allow either the cell-division or turnover of GC B cells to vary with cell-age.

I have fitted the age-structured model on GC cells and it fails to fit to LN GCs and show Δ AIC of 380 in spleen GCs.

We explored the possibility of kinetically heterogeneous subpopulations within the GC compartment that divide and turnover at different rates (as discussed in FM B cells section). The model of kinetic heterogeneity despite being over-parametrised and complex, fails to improve on the fits from the simple homogeneous model (ΔAIC = 0 and 2 for spleen and LN GCs respectively, Table 4). Additionally, the estimates for net loss rates for the two subpopulations were similar (λ_1 = 0.03(0.01, 0.07) and λ_2 = 0.09(0.03, 0.26) for spleen GC cells and λ_1 = 0.02(0.01, 0.03) and λ_2 = 0.05(0.01, 0.23)for LN GC cells) and almost equal to the estimates from the simple homogeneous model (λ = 0.06(0.04, 0.08) for spleen GCs and λ = 0.02(0.01, 0.03) for LN GCs). We also addressed the possibility that host and donor cells may behave differently due to the presence of a persistent incumbent subpopulation in the host compartment [Hogan et al PNAS 2015, Rane et al PLoS Bio 2018] that may arise during neonatal stages as the early waves of mature B cells begin to populate the periphery. The incumbent model also failed to explain GC B cell dynamics giving visually poor fits to the timecourse of normalised donor fractions (not shown) and received lowest statistical support (Table 4).

Our analysis of FM population dynamics shows that the total size of FM compartment increases with time, due to a gradual decline in their turnover (Figure 1A, left panel). We tested whether the rate of influx of FM cells into the GC compartment also varies with host-age, so as to maintain the inflow of constant number of cells over time. We found that allowing the rate of influx to vary with host-age, results in very poor descriptions of the normalised donor fractions in both, spleen and LN GC populations (Δ AIC of 385 and 355 respectively) and hence rejected this possibility. Therefore, follicular cells maturing into the GC compartment at any given time are proportional to the size of the FM population at that moment.

GC B cells maintain high Ki67 expression by active division and rapid turnover.

As described earlier for FM cells, we used an additional validation strategy to test the robustness of the model with the best-fit to cell counts and normalised donor fractions, by comparing its predictions of $Ki67^{hi}$ fractions to the observed data. GC populations in both, spleen and lymph nodes, are always highly enriched for recently divided cells with more than 90% cells expressing high levels of Ki67 throughout the timecourse that we studied. We analysed the transition of GC cells between $Ki67^{hi}$ and $Ki67^{lo}$ subsets using the model described in eq. 3, separately for the host and donor compartments. The rate of influx of FM cells into the GC compartment changes very slowly (it takes $\approx$ a year for the number of FM cells maturing into GC compartment to fall by

a factor of two), which allows us to assume that the Ki67^{hi}and Ki67^{lo}GC subsets are in the state of quasi-equilibrium. We could then simulate the model described in eq. 3 using the rates of cell-division and turnover of Ki67^{hi}and Ki67^{lo}subsets inferred from the estimates of mean clonal lifetimes (Table 5) of splenic and LN GC cells (detailed description in methods and Hogan et al. PNAS 2015).

The simple model with constant rates of cell-division and turnover captured the trend in Ki67^{hi}fractions in both, host and donor compartments of splenic and LN GC populations remarkably well (Figure 2 C). These predictions were made by assuming that the recently divided cells lose their post-mitotic Ki67 expression by $\approx$ 3.5 days [Gossel et al., eLife 2017] and also that the cell-division event does not alter the ability of cells to persist in GC compartments. Since, majority of GC precursors (FMs) have fewer Ki67^{hi}cells ($\simeq$ 10%), we speculate that almost all of the GC cells undergo multiple rounds of cell-division or are lost from the pool rapidly before losing their Ki67 expression or both.

Summary/Discussion

- GC cell dynamics vary between spleen and lymph nodes, as observed by slower replacement kinetics of LN GC cells as compared to their splenic counterparts. Accordingly, our analysis predicts longer persistence of GC cells in lymph nodes than in spleen.
- Simple homogeneous model with constant rates of cell-division and turnover explains GC cell dynamics and predicts the kinetics of Ki67^{hi}fractions remarkably well, in both spleen and lymph nodes. This may suggests that under the influence of very strong antigen derived signals the mean inter-division and residence times of all cells within the GC pool, remain constant across different instances of germinal centre reactions, over time. The strong signals from antigen and Th cells wash out the time-variability in turnover of their precursors (FMs)?
- Prolong GC reactions in response to viral antigens and gut microbes (Adachi et al. 2015, Bachman et al. 1996, Kasturi et al. 2011) may allow B cells to reach higher degree of affinity maturation $\rightarrow$ means to cope with constant antigenic drifts. An important question is whether chronic GC response consists of long-lived GC cells or constant invasion of short lived cells maintaining a long-lived steady state? Our analysis favours second scenario.
- Ki67 in GC is result of active division and turnover and is not source derived. FM population has 10% Ki67^{hi}cells while GC are $\sim$ 95% Ki67^{hi}.

	Model and ΔAIC			
population	Simple homogeneous	Time-dependent	Kinetic heterogeneity	Incumbent
Spleen GC cells	0	2	0	310
LN GC cells	2	5	0	250

Table 4: **Comparison of AIC values for different models fitted to cell counts and donor fractions in spleen and LN GC B cells normalised to the chimerism in FM cells (spleen + LN).**

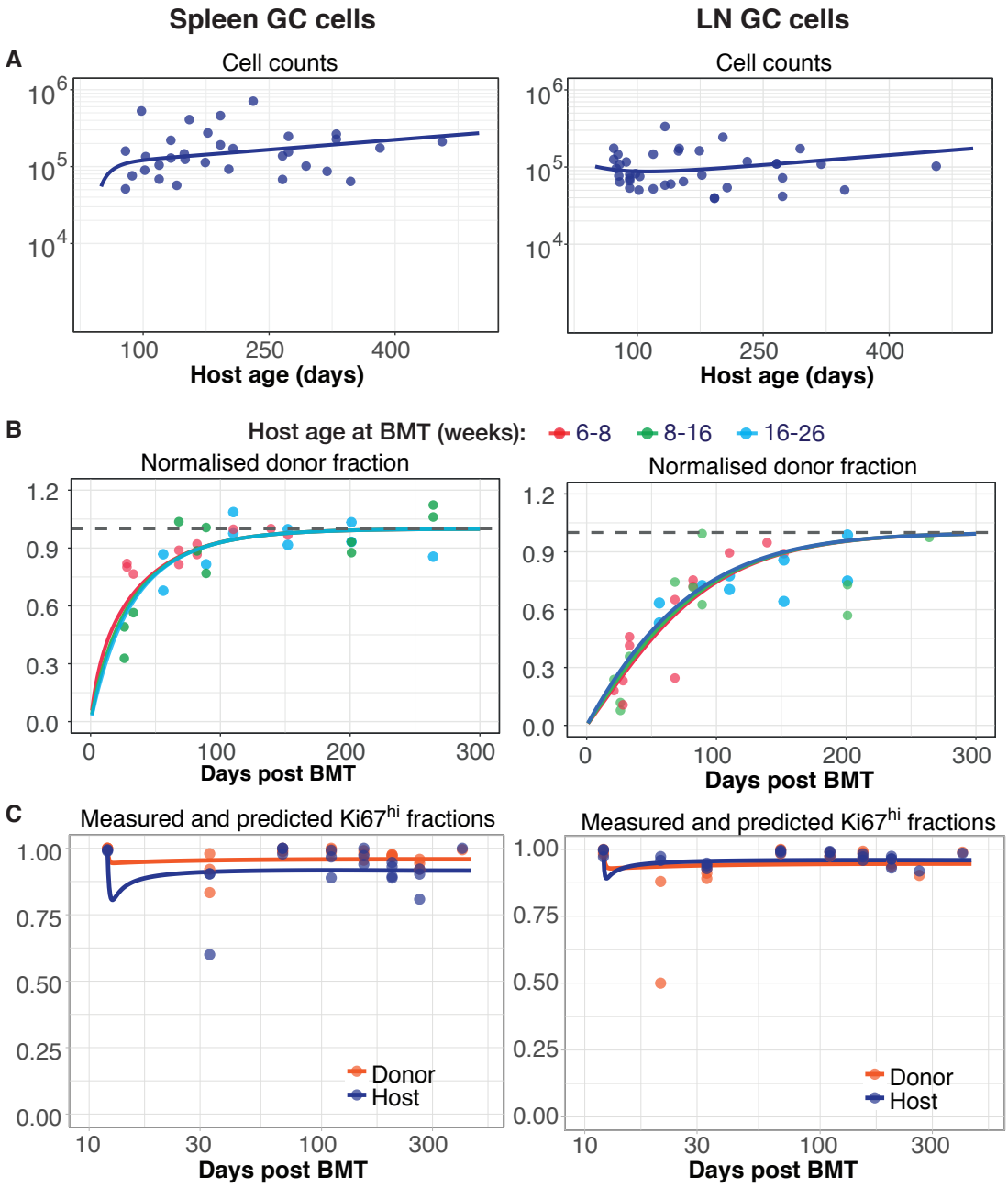

Figure 2: **Fitted and predicted population dynamics of spleen and LN GC B cells, using the best-fitting model in which cells the net loss rate of cells remain constant with time.** (A) The model was fitted simultaneously to the extended time-courses of total cell counts of spleen and LN GC B cells pooled in busulfan chimeras, and their donor fractions normalised to the chimerism in FM B cells. The latter took account of different ages at BMT (right-hand panel, coloured lines; each generated using the mode of the age within each group). (C) We then used the model parameters to predict the proportion of cells that were Ki67^{hi} within host and donor spleen and LN GC B cells over time.

Population	Parameter	Estimate	95% CI
Lymph node GC cells	Total cell numbers at age 7 wks ($\times 10^{-3}$)	104	(46, 230)
	Total daily influx from FM into LN GC at age 7 wks ($\times 10^{-3}$)	1.8	(1.3, 2.4)
	Mean clonal life-time (days)	41	(31, 64)
Splenic GC cells	Total cell numbers at age 7 wks ($\times 10^{-3}$)	10.5	(0.15, 735)
	Total daily influx from FM into spleen GC at age 7 wks ($\times 10^{-3}$)	6.6	(4.7, 9.1)
	Mean clonal life-time (days)	15	(12, 21)

Table 5: **Parameter estimates from the best-fit (simple homogeneous) model for spleen and LN GC B cells.** Confidence intervals were estimated using the inverse of the Hessian matrix at the ML estimates of the parameters.

Methods

Predicting the kinetics of Ki67 expression in host and donor FM cells

We divide host and donor cells into recently divided (Ki67^{hi}) and quiescent (Ki67^{lo}) compartments (Figure 3).

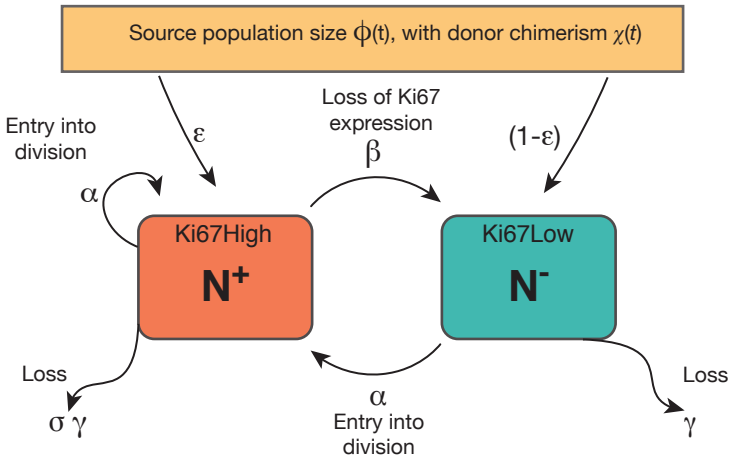

Figure 3: Two compartment model for prolifeartion and loss of B cells using Ki67 as a marker for dividing and recently divided cells

This system is represented by the following coupled ordinary differential equations:

$$\begin{aligned} \frac{dN^+}{dt} &= \epsilon\,\phi(t) + \alpha\,(2\,N^- + N^+) - \beta N^+ - \sigma\,\gamma N^+, \\ \frac{dN^-}{dt} &= (1-\epsilon)\,\phi(t) - \alpha N^- + \beta N^+ - \gamma N^+. \end{aligned} \tag{3}$$

Here,

- ϵ denotes the proportion of the cells entering from source that are Ki67^{hi}. Estimated from the data ($\simeq$ 0.8 for T2s and 0.1 for FMs).
- γ is the rate of loss (death + maturation) of Ki67^{lo} cells.
- In general it is possible that recently-divided Ki67^{hi} cells may have different susceptibility to loss than quiescent (Ki67^{lo}) cells. The parameter σ is the relative loss rate of Ki67^{hi} and Ki67^{lo} cells. In the simulations we ran we assumed $\sigma = 1$.
- α is the rate of entry into division for both Ki67^{lo} and Ki67^{hi} cells. The mean inter-division time is $1/\alpha$.
- β is the rate of loss of Ki67 expression after mitosis. We and others have estimated the mean expression time $\frac{1}{\beta}$ to be 3.5 days.

The quantities $\phi(t)$ and $\chi(t)$, are obtained from splines fitted to the numbers of host and donor cells in the source populations.

The total size of GC population is then derived by summing up Ki67^{hi}and Ki67^{lo}subsets from equation 3.

$$\begin{aligned} \frac{dN}{dt} &= \phi(t) + \alpha\, N - \gamma\, N\, (\sigma\, \kappa + 1 - \kappa) \\ \frac{dN}{dt} &= \phi(t) + \alpha\, N - \gamma\, N \qquad\qquad \text{for } \sigma = 1, \end{aligned} \tag{4}$$

where κ is the fraction of Ki67^{hi}cells in the given population.

(i) For FM cells, we compare the equations 1 and 4, which shows that $\gamma = \delta_0\, e^{-r\, t}$ and $\alpha = \rho$. We use the estimates of δ_0, r and ρ obtained from the fits to cell counts and normalised donor fractions.

(ii) For GC cells we calculate α and γ using the estimate of net loss rate λ obtained from the fits to the cell counts and normalised donor fractions, as described in [Hogan et al., PNAS 2015].

The influx from the source population changes very slowly relative to the rates of flow between Ki67^{hi} to Ki67^{lo} and so we assume that these populations (N^+ and N^-) are in quasi-equilibrium. (This means they are locked into the kinetics of the source population and don't slosh around as the influx is changing. Think about being able to keep your balance standing on a moving train – if it's not too jerky you can keep stably upright because the timescale over which your muscles can respond is pretty quick. If the train accelerates or decelerates fast, you will respond by lurching around a bit before you regain equilibrium).

We then solve the coupled ODE described in eq. 3 numerically using initial numbers of Ki67^{lo} and Ki67^{hi} host and donor cells taken from the experimental obervations at t_0 = 12 days post BMT. We solve them twice – once for host cells with influx $\phi(t)(1 - \chi(t))$, and again for donor cells with influx $\phi(t)\chi(t)$. We then calculate the predicted Ki67^{hi} proportions $N^+/(N^+ + N^-)$ in host and donor cells over time.