Population dynamics of B cell subsets – analysis and predictions

Follicular Mature B cells

We aimed to quantify the population dynamics of follicular mature (FM) B cells and to understand their rules of replacement. We adopted the conventional view of B cell development and assumed that Transitional 2 (T2) cells are the direct precursor of FM cells. 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 modeling their dynamics.

One concern: Sanket tried T1 as source and it actually gives better fits in all FM cases (ΔAIC $\simeq$ 5). But we are going with dogma and saying T2 is source. It's puzzling to us that there is apparently so much residual Ki67 expression in FM from the T2 source, if all the division is pre-T1 as you say. Makes me worry a littl...

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

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

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.

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

The net loss rate λ dictates the rate of replacement of FM 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 (Figure 1B) 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 subpopulations 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 time-courses 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.

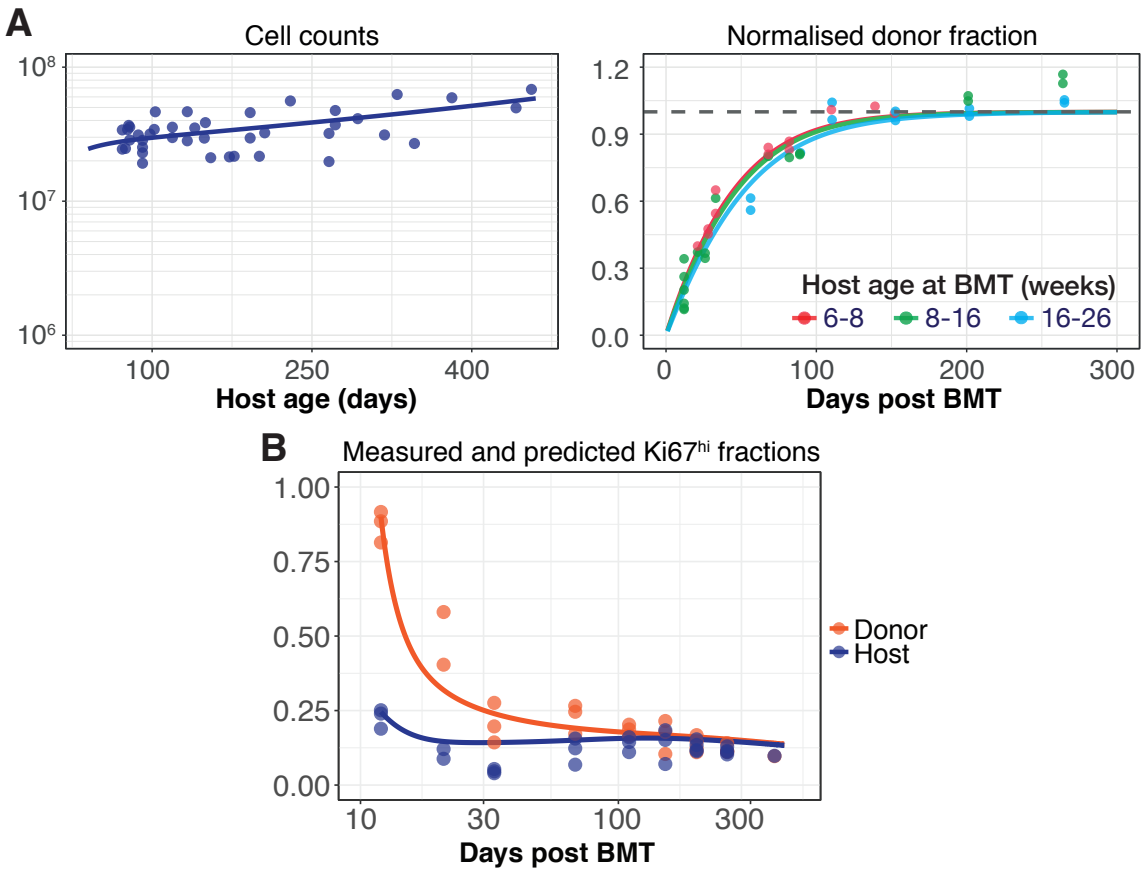

Figure 1: **Fitted and predicted population dynamics of FM B cells, using the best-fitting model in which cells divide at a constant rate and their mean residence time increases with host age.** (A) The model was fitted simultaneously to the extended timecourses of total cell counts of FM B cells pooled 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.

The simulations show that the host/donor disparity in Ki67 expression derives largely from residual expression 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 $\triangle$AIC				
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)

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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 $\approx$ 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	△AIC	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 the level of their source (Figure 2 B), suggesting that the rules of replacement are identical for host and donor cells. Interestingly, the turnover of GC B cells in the spleen is more rapid than in lymph nodes, with the chimerism 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 kinetics of infiltration 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 used the similar approach as that of FM cells and modelled the behaviour of actively dividing and short-lived GC B cells by allowing their net rate of loss λ to vary with host-age. The kinetics of cell counts and donor fractions in spleen and LN GC cells was described well visually by the model in which turnover δ varies with host-age, while varying the rate of cell division ρ yields un-physiological parameter estimates when fitted to the data. Interestingly, we found that changes in δ with host age were extremely slow such that the time taken for the mean residence time of spleen and LN GC cells to double is 3900 and 2500 days, respectively. Therefore, at least up to 1 year of age (within the time-line of these experiments), we find no strong effect of age associated variations in the host environment on the turnover of B cells participating in GC reactions, in mice.

Our analysis from the time-dependent model suggested that the population dynamics of GC B cells can be explained by rates of division and turnover that do not vary substantially with host age. So, we fitted the time-course of cell counts and donor fractions simultaneously using the simple model where the rate of net loss λ, which is the net effect of cell-division and loss, stays constant with host-age (Figure 2 A and B). We find relatively stronger statistical support for the constant birth-death model, in both spleen and LN GC population, as compared to the model where λ varies with host-age (Δ AIC = 2 and 3, respectively). Consistent with the disparity in donor fraction kinetics in spleen and lymph nodes, the mean life-time of a clonal lineage 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). 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.

?No heterogeneity or weak heterogeneity?

Each instance of GC reaction lasts for about 3-4 weeks, which makes it highly unlikely for the turnover or division of GC B cells to be strongly influenced by the cell-intrinsic changes accumulated over such a short period of time. Additionally, rapid kinetics of inflation and collapse of GC clusters suggest that majority of GC cells may be short-lived with lifetimes much shorter than 3 weeks. Therefore, we refrained

from using highly complex models of cell-age associated changes in either turnover or division of GC B cells.

We explored the possibility that the GC B cell population is composed of kinetically heterogeneous subsets (as discussed in FM B cells section) but found that this model despite being over-parametrised and complex, does not improve on the fits from constant birth-death model (Table 4). Additionally, we find no strong evidence for kinetic heterogeneity within the GC population using this model (λ_1 = 0.03(0.01, 0.07) and λ_2 = 0.09(0.03, 0.26) for spleen GC cells and λ_1 = 0.05(0.01, 0.23) and λ_1 = 0.02(0.01, 0.03) for LN GC cells). Alternatively, varying the rate with which follicular cells participate in GC reactions, while keeping the net cell loss constant also fails to capture the kinetics of cell counts and normalised donor fractions in spleen and LN GC populations (Δ AIC of 385 and 355 respectively).

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 also addressed the possibility that host and donor cells may behave differently due to the presence of a persistent incumbent subset 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 fails to explain the GC B cell dynamics giving visually poor fits to the normalised donor fraction (not shown) and received lowest statistical support (Table 4).

Dynamics of ki67 expression in the actively dividing GC B cells.

As described earlier for the FM B cells, we used an additional validation strategy to test the robustness of the best-fitting model by comparing its predictions of the kinetics of Ki67^{hi}fractions for the GC B cell population to the observed data. We assumed that the Ki67^{hi}and Ki67^{lo}subsets are in the state of quasi-equilibrium, since the rate of change of source influx is much slower than the rate of loss of ki67 expression. This allowed us to infer the rates of turnover 'δ' and division 'ρ' from the estimates of total net loss rate λ obtained by fitting the constant birth-death model to the time-course of cell counts and normalised donor fractions in GC cells (detailed description in methods and Hogan et al. PNAS 2015).

In both spleen and LN GC B cell populations, the fraction of Ki67^{hi}cells was continuously maintained high ($\sim$ 0.9), over a year post-BMT. We found that the best-fitting constant birth-death model predicts this trend extremely well for donor and host compartments in both tissues (Figure 2 C). To amke these predictions we asumed that the mean lifetime of ki67 expression post mitosis was 3.5d and also considered that Ki67^{hi}cells die or differentiate at an equal rate as that of Ki67^{lo}cells. Majority of the follicular cells participating in GC reactions have low ki67 expression (Ki67^{hi}fraction in FM $\approxeq$ 0.1), suggesting that almost all of the GC cells undergo multiple rounds of cell-division or are lost from the pool rapidly before losing ki67 expression or both.

Summary

- Gc cell dynamics vary between spleen and lymph nodes, as seen by slower replacement kinetics and longer clonal life-times of LN GC cells.
- Simple homogeneous model with constant lambda successfully ecplains GC cell dynamics. 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 center reactions, over time. The strong signals from antigen and Th cells washes out the heterogeneity between GC cell precursors? This is particularly intersting since there are reports of dark and light zones in GC reactions that are apparent roughly around 10days of GC lifetime. Could be the result of delayed division due to selection and affinity maturation events? I am not sure if we are capturing true GC dynamics here.

- Ki67 in GC is result of active division and turnover and is not source derived. FM population has 15% Ki67^{hi}cells while GC are $\sim$ 95% Ki67^{hi}.

	Model and $\triangle$AIC			
population	constant birth-death model	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).**
* No effect of host-age on the net loss rate λ makes the time-dependent model equivalent to the constant birth-death model. † No difference between the kinetics of heterogeneous subsets.

Population	**Parameter**	Estimate	95% CI
Lymph node GC cells	Total cell numbers at age 7 wks ($\times 10^{-3}$)	104	(46, 230)
	Total daily of influx from T2 into FM at age 7 wks ($\times 10^{-3}$)	1.8	(1.3, 2.4)
	Mean clone life-time (days)	41	(31, 64)
Splenic GC cells	Total cell numbers at age 7 wks ($\times 10^{-3}$)	3.7	(4.1, 3500)
	Total daily of influx from T2 into FM at age 7 wks ($\times 10^{-3}$)	6.4	(4.6, 8.9)
	Mean clone life-time (days)	15	(12, 21)

Table 5: **Parameter estimates from the best-fit (constant birth-death) 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.

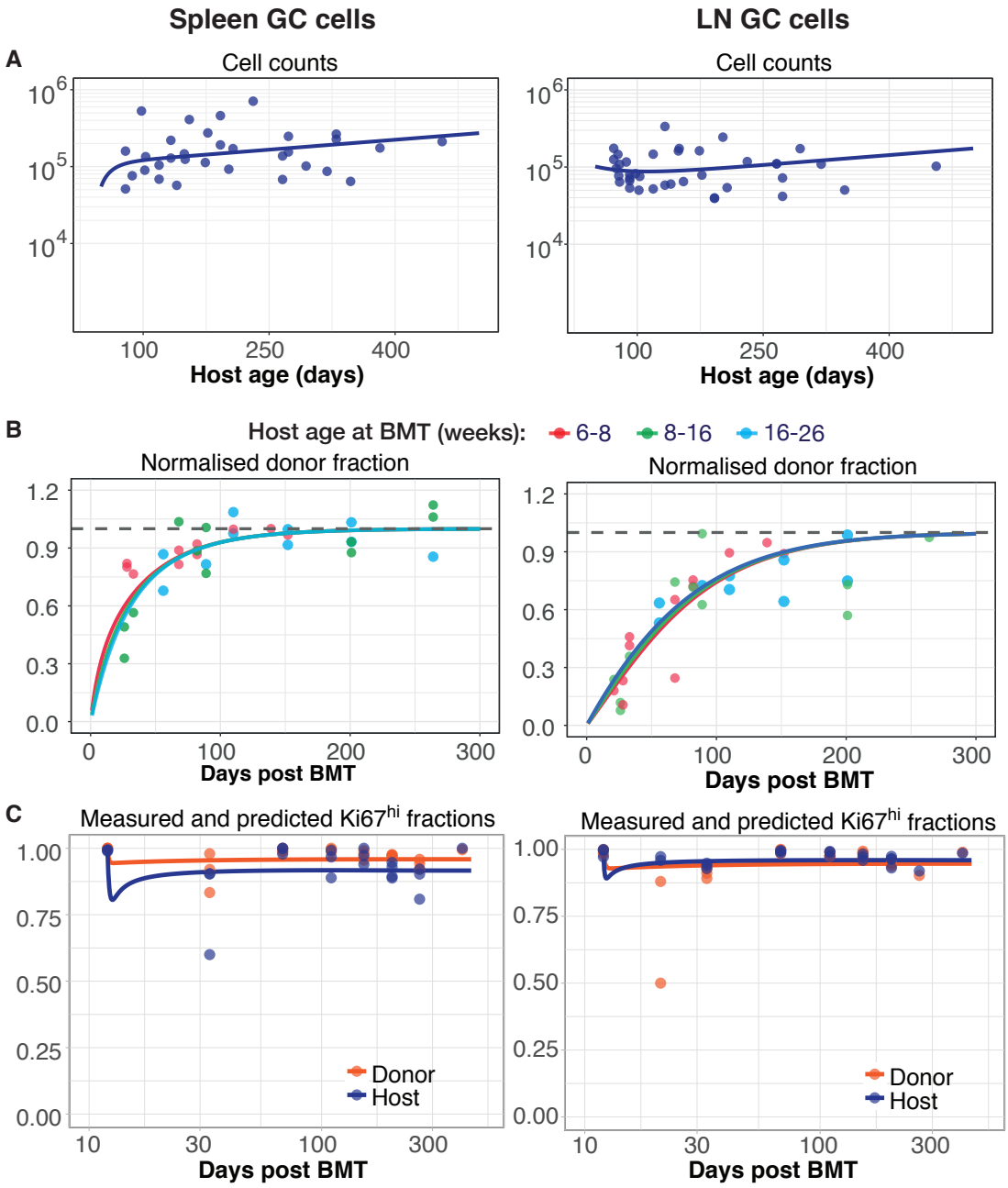

Figure 2: **Fitted and predicted population dynamics of spleen and LN GC B cells, using the best-fitting model in which cells the net loss rate of cells remain constant with time.** (A) The model was fitted simultaneously to the extended time-courses of total cell counts of spleen and LN GC B cells pooled 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.

Methods

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

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

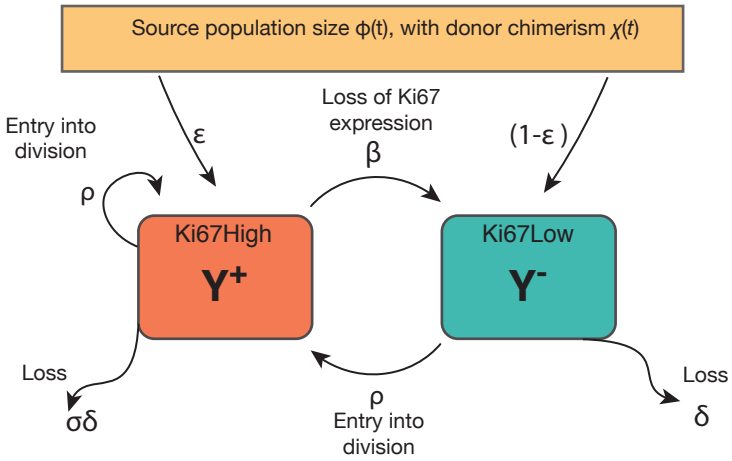

Figure 3: Two compartment model for prolifeartion and loss of B cells using Ki67 as a marker for dividing and recently divided cells

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

$$\begin{aligned} \frac{dY^+}{dt} &= \epsilon\phi(t) + \rho\,(2\,Y^- + Y^+) - \beta Y^+ - \sigma\delta Y^+, \\ \frac{dY^-}{dt} &= (1-\epsilon)\phi(t) - \rho Y^- + \beta Y^+ - \delta Y^+. \end{aligned} \tag{2}$$

Here,

- ϵ denotes the proportion of the cells entering from T2 that are Ki67^{hi}. Estimated from the data ($\simeq 0.8$).
- $\chi(t)$ is the time-varying chimerism of the source, estimated from the data and averaged over all mice.
- δ 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 $\sigma = 1$.
- ρ is the rate of entry into division for both Ki67^{lo} and Ki67^{hi} cells. The mean interdivision time is $1/\rho$.
- 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 ϕ and $\chi(t)$, are obtained from splines fitted to the numbers of host and donor cells in the source populations, and $\delta(t)$ and ρ come from the fits to cell counts and donor fractions within FM. The influx from the source population (T2) changes very slowly relative to the rates of flow between Ki67^{hi} to Ki67^{lo} and so we assume that these populations (Y^{+} and Y^{-}) 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 equations above with initial numbers of Ki67^{lo} and Ki67^{hi} host and donor cells taken from the experimental obervations at t_0 = 21 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 $Y^{+}/(Y^{+}+Y^{-})$ in host and donor cells over time.