Population dynamics of Follicular mature and germinal center B cells

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.

The busulfan chimera system allows us to follow the fate of newly made B cells as they develop and differentiate into mature B cell subsets. The kinetics with which new (donor-derived) B cells percolate into peripheral B cell subsets and displace older host-derived cels allow us to map their differentiation pathways, quantify their homeostatic dynamics, and determine the rules of replacement within each subset.

To help constrain the model fitting and parameter estimation, we used observations from Ki67-CreER-YFP reporter mice to put priors on the net rates of loss of FM and GC B cells in spleen and LN. We measured the frequencies of YFP-expressing cells in these subsets 15 and 66d after the transient induction of YFP expression in cells expressing Ki67 (Figure). Given that the compartments may also accumulate YFP+ from their upstream precursor populations during this period, the decline in YFP expression each subset yields approximate upper bounds on the net rate of loss of cells in each compartment put these numbers on the YFP figure.

Follicular Mature B cells are a homogeneous, slowly dividing population whose lifespan increases with age

We generated timecourses of numbers and Ki67 expression levels of host and donor cells within the FM B cell pool in mice after the establishment of stable bone marrow chimerism in mice. To allow us to examine any impact of age on homeostatic dynamics, we generated chimerism in mice of ages spanning ?? and ??? weeks, and followed them out to a year of age. We wanted to use this information to assess the relative support for a variety of different models of FM B cell homeostasis, shown schematically in Figure 1. We assumed that FM cells circulate freely in the lymphatic system CITE , and so we pooled the numbers of these cells recovered from spleen and lymph nodes when modelling their dynamics.

In all models we assumed that cells differentiate into FM B cells at a rate proportional to the size of their precursor population, which assumed could be either T1, T2, or T1 and T2 combined. The simplest model (Figure 1A) assumed that FM B cells, whether host or donor, form a homogeneous population in which all cells are lost through death or differentiation at the same rate δ and self-renew through division at the same rate ρ. However the approach to stable chimerism appeared to initially be rapid and then gradually slow (Figure 2B). To detect and describe any multiphasic behaviour we considered three extensions to this basic model. In the first, the rate of turnover (δ) or division (ρ) might vary with host age (the 'time dependent' model, Figure 1B). In the second, FM B cells comprise two independent subpopulations turning over at different rates (Figure 1C). In this we would expect the donor chimerism initially to increase rapidly as the subpopulation with faster turnover is replaced, followed by a more gradual approach to stable chimerism as the more persistent subpopulation, with slower turnover, is replaced. Third, we considered a model in which the FM pool is

homogeneous with constant rates of division and turnover, but fed from the source population at a rate that declines with age (Figure 1D), which may also explain any decelerating approach to stable chimerism.

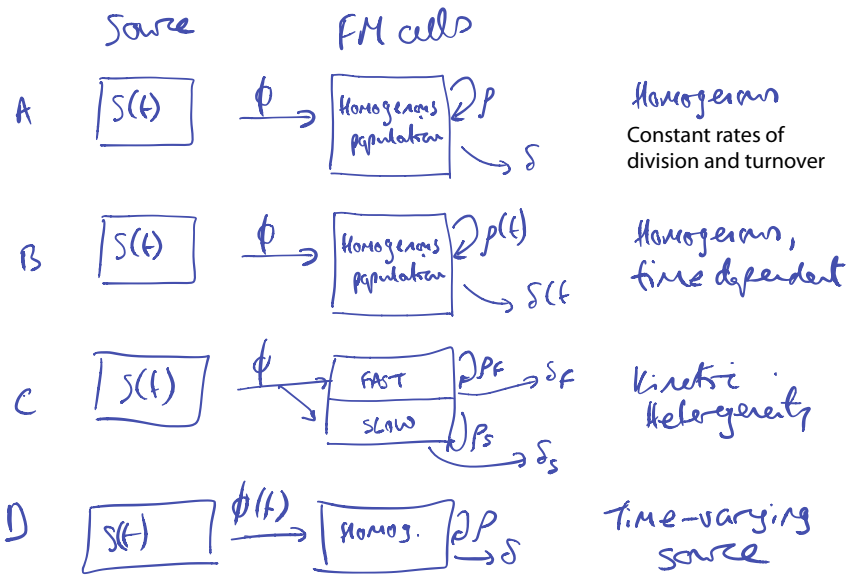

Figure 1 – Schematic descriptions of the candidate models of FM B cell homeostasis

We fitted each model simultaneously to the timecourses of the total size of the FM pool, the chimerism within FM B cells normalised to that in T1 (the earliest common precursor to all populations considered), and the proportions of host and donor FM cells expressing Ki67. We found strongest support for the model in which the loss or turnover rate δ changes with host age and the division rate ρ remains constant, with T1 transitional cells as the direct precursor of FM B cells. Fits and data are shown in Fig. 2; see Methods for the mathematical formulation of the models and the fitting strategy. The measures of relative support for this and the alternative models are given in Table 1.

Figure 2 – **Population dynamics of FM B cells in busulfan chimeric mice**, using the best-fitting model in which cells divide at a constant rate and their mean lifespan increases with host age. Solid lines denote the most probable simultaneous description of the observations of (A) cell counts, (B) the chimerism within FB cells normalised to that in T1, and (C) the proportions of host and donor cells expressing Ki67. Shaded envelopes indicate the effect of uncertainty in parameters on the model fit, generated by drawing samples from the posterior distribution of parameter estimates and shading within the 4.5 and 95.5 percentiles of the resulting model predictions. Different colours in (A) and (B) indicate the model fits for mice grouped according to the age at which they underwent bone marrow transplant (BMT). Model predictions were generated using the mean age at BMT within each group.

We estimate that FM B cells have a mean lifetime of roughly 34 days in 7 week-old mice, and that this life expectancy doubles on an average every 28 months. While levels of Ki67 in FM B cells are appreciable (approximately 10%; Fig. 2C), we find that the bulk of this derives from newly generated FM cells who inherit it from the highly proliferative T1 population; roughly 4% of FM B cells are replaced each day by new immigrants and indeed the donor-derived FM B cells, which soon after BMT are highly enriched for newly generated cells, show significantly higher levels of Ki67 than host cells (Fig. 2C). We infer that FM B cells

	Model and ΔLOO-IC			
Source	Time-dependent	Simple homogeneous	Kinetic heterogeneity	Incumbent
T1	0	8	7	9
T2	36	43	39	42
T1 + T2	19	29	28	29

Table 1 – Comparison of models describing the population dynamics of Follicular Mature (FM) B cells, pooled from LN and spleen. switch to model weights, or add them LOO-IC values obtained using leave-one-out cross validation method 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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themselves self-renew infrequently, with an estimated mean interdivision time of approximately 9 months. Because this self-renewal is slow, the average lifetime of a clone (derived from the net rate of loss and proliferative renewal, $\delta - \rho$) is close to the life expectancy of individual cells. Parameter estimates and 95% credible intervals are in Table 2.

Parameter	**Estimate**	**95% CI**
Percent daily replacement by source at age 7 wks	3.9	(3.2, 13)
Mean clonal lifetime (days) at age 7 wks	38	(30, 48)
Mean residence time (days) at age 7 wks	34	(26, 42)
Mean inter-division time (days)	268	(120, 1600)
Time for mean residence time to double (months)	28	(15, 180)
Average time for $Ki67^{hi}$ cells to transition to $Ki67^{lo}$ (days)	6.0	(4.6, 7.3)

Table 2 – Parameter estimates from the best-fitting model of FM B cell homeostasis. 95% credible intervals were estimated by taking the 2.5 and 97.5 percentiles of the posterior probability distribution of the parameter values.

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 rates of loss or division of FM cells with cell age.

Germinal Center B cells

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

We took a similar approach to modelling Germinal Center (GC) B cells. However, in contrast to FM B cells, we observed that the chimerism of GC B cells in the spleen stabilised roughly twice as fast as that in lymph nodes (∼110 and ∼260 days post-BMT, respectively). This disparity in kinetics led us to model GC cell homeostasis in spleen and lymph nodes separately.

We assumed a constant rate of source influx in both spleen and LN GC pools and allowed our models to be strongly informed by the rate of turnover (λ) observed in Ki67-CreER-YFP reporter mice. Additionally, we found that recently divided YFP-tagged splenic GC cells were lost twice as fast as LN GC cells in Ki67-CreER-YFP reporter mice (half life 11 vs 20 days respectively, details in the Methods?).

Spleen GC cells in the spleen are homogeneous, derived directly from transitional B cells, and their longevity increases with the age of the host

Total numbers of spleen GC cells increased with age but the proportions of cells expressing Ki67 remained constant, and were high and almost identical for donor and host cells (Figure 3A and C). These observations suggest either a gradual increase in the rate of generation of new splenic GC B cells from their precursor population, or an increase in GC B cell lifespan with age. The chimerism of splenic GC B cells stabilises more rapidly than that of FM B cells (roughly 110 days vs. 210 days post BMT), meaning it is unlikely that FM B cells are their direct precursor. We infer that splenic GC B cells are instead sourced directly from the transitional (T1 and/or T2) B cell subsets. These empirical observations therefore limit the set of plausible descriptive models of splenic GC B cell homeostasis.

Using this information and, as before, fitting each model simultaneously to the numbers, chimerism and Ki67 expression levels of spleen GC B cells, we found that the model of time-varying loss with T2 B cells as their immediate precursor received strongest statistical support (Table 3; fits shown in Fig. 3). We find that spleen GC B cells are far more dynamic than FM B cells, with lifetimes and mean interdivision times of roughly half a day. The net effect of these processes yields a mean lifespan of spleen GC B cells of about 15 days in 7 week old mice (Table 4). We infer that the expected lifespan of individual GC B cells doubles roughly every 8 months. We also infer that the maintenance of splenic GC B cell numbers is highly dependent on immigration, with approximately – and remarkably – 36% of the population replaced by the cells from the circulatory T2 compartment every day.

Lymph node GC B cells comprise a mixture of short-lived and persistent clones and likely derive from FM B cells

As in the spleen, GC B cells in lymph nodes (LN) increase in numbers with age and donor and host cells express Ki67 at similar and high levels (Fig. 4A and C). The numbers, chimerism and Ki67 expression of GC B cells in LN were best explained by a model invoking two subsets of cells with different kinetics, sourced by FM B cells (Fig. 4). This model was favoured with a weight of XYZ% relative to all other models considered (Table 3).

Our analysis reveals that some GC clones are very short-lived, being lost from the lymph nodes within days (average clonal lifespan of $\sim$ 5 days, Table 5) while others persist for on average 2 months (Table 5). In a

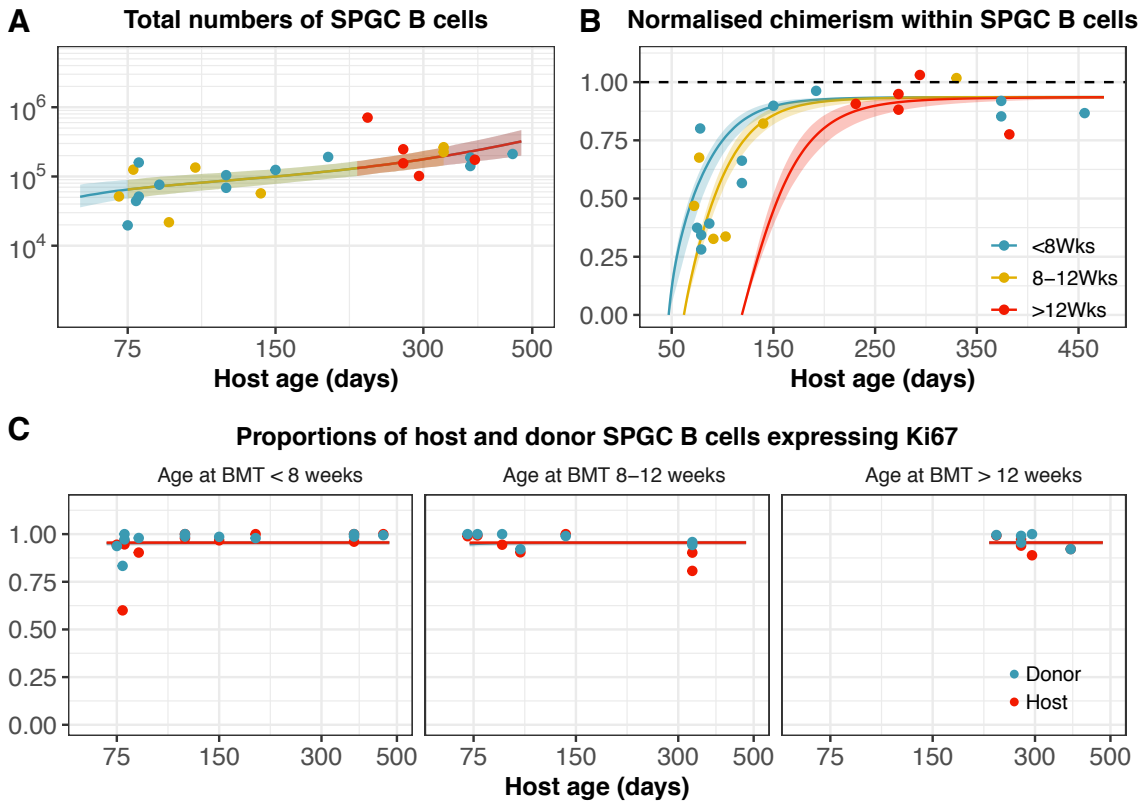

Figure 3 – **Population dynamics of Spleen GC B cells in busulfan chimeric mice**, using the best-fitting model in which cells divide at a constant rate, their mean lifespan increases with host age, and T2 B cells are their precursor population. Note that the chimerism normalised to T1 stabilises at a value <1, equal to the chimerism in T2 normalised to T1. Solid lines denote the best-fitting model predictions of the timecourses of (A) cell counts, (B) normalised chimerism donor fractions and (C) Ki67^{hi}fractions. Prediction intervals (4.5th and 95.5th percentiles) were generated by drawing samples from the posterior distribution of parameter estimates. Different colours in (A) and (B) indicate mice grouped according to the age at BMT. Predictions for each group were drawn using the mean age at BMT within each group.

		Model and ΔLOO-IC			
Location	Source	Simple homogeneous	Time-dependent	Kinetic heterogeneity	Incumbent
Spleen	T1	17	9	33	12
	T2	10	0	33	9
	FM	13	8	32	9
Lymph nodes	T1	55	41	12	54
	T2	50	42	12	51
	FM	12	35	0	38

Table 3 – **Comparison of models describing the population dynamics of Germinal center B cells in spleen and lymph nodes**. Model weights were calculated using the Leave-one-out information criterion (see Methods)

Parameter	Estimates and 95% CI
Rate of influx from precursors ($\times 10^{-3}$ cells/day)	4.7 (3.2, 6.8)
Mean clonal lifespan at age 7 weeks (days)	15 (13, 16)
Mean cell lifespan at age 7 weeks (days)	0.46 (0.34, 0.61)
Mean inter-division time (days)	0.48 (0.35, 0.64)
Time taken for τ to double(months)	7.9 (4.5, 25)
Average time for $Ki67^{hi}$ cells to transition to $Ki67^{lo}$ (days)	5.2 (3.8, 6.7)

Table 4 – Parameters governing homeostasis of GC B cells in the spleen. Estimates from the best-fitting model, in which thre longevity of splenic GC B cells increases with host age, and T2 cells are their direct precursor. 95% credible intervals were estimated using the 2.5 and 97.5 percentiles of the posterior distributions of the parameter values.

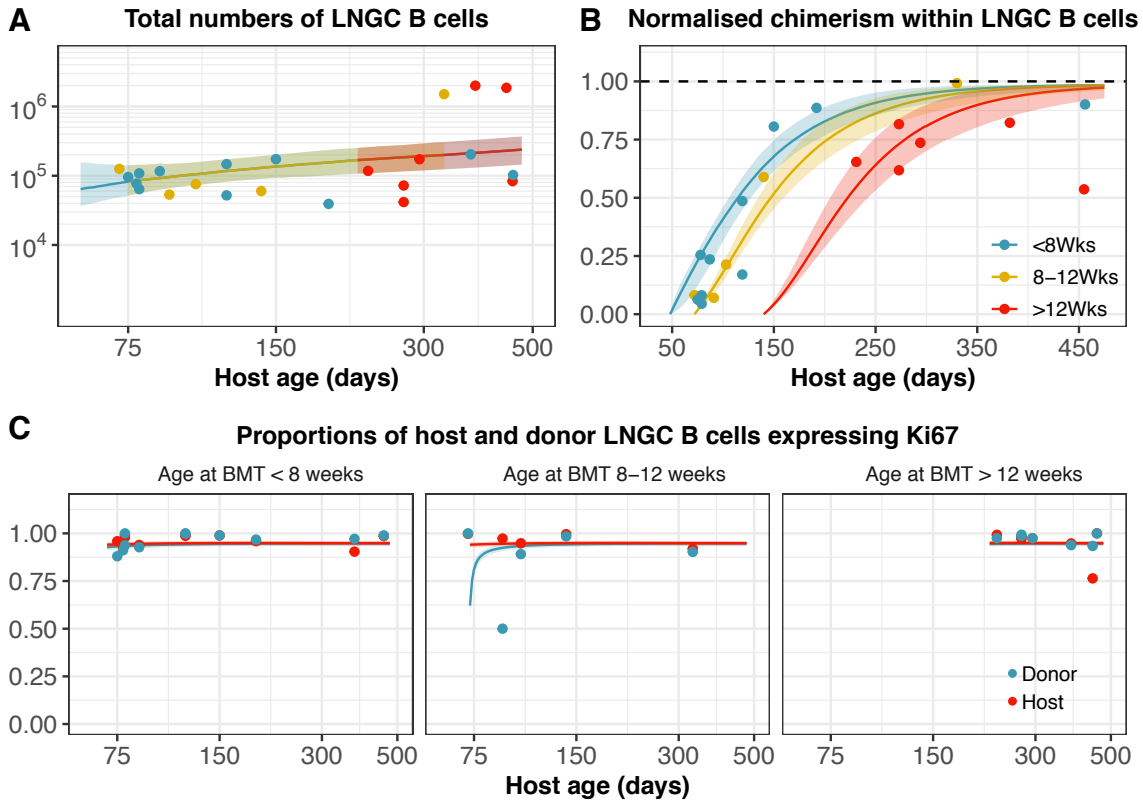

Figure 4 – Population dynamics of Lymph node GC B cells in busulfan chimeric mice, using the best-fitting model of kinetic heterogeneity with FM B cells as the source. See Fig. 3 for details of fitting procedure.

7 week old mouse, we infer that the LN GC compartment contains short and long-lived clones in roughly equal proportions. We also find that roughly 25% LN GC cells are replaced daily by new cells derived from the recirculating FM B cell pool.

Parameter	**Estimates and 95% CI**	
	Transient Subset	Persistent Subset
Rate of influx from precursors ($\times 10^{-2}$ cells/day)	7.0 (0.2, 40)	24 (12, 43)
Mean cell lifespan (days)	0.69 (0.42, 1.3)	0.90 (0.46, 1.9)
Mean clonal lifespan (days)	5.0 (1.2, 19)	68 (34, 130)
Mean inter-division time (days)	0.91 (0.47, 2.0)	0.79 (0.46, 1.9)
Proportion of population at age 7 weeks	0.51 (0.17, 0.72)	0.49 (0.28, 0.83)
Average time for $Ki67^{hi}$ cells to transition to $Ki67^{lo}$ (days)	5.1 (3.7, 6.6)	5.1 (3.7, 6.6)

Table 5 – **Parameter estimates for LN GC B cell homeostasis**, derived from the best-fit (kinetic heterogeneity) model with FM B cells as the precursor population. Credible intervals were estimated by taking 2.5^{th} and 97.5^{th} percentiles of the posterior probability distribution of the parameter values obtained after fitting model to the data.

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 a large fraction of GC cells in lymph nodes than in spleen.
- Heterogeneity in LNGCs stems from pooling multiple LNs?
- 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?
- 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% $Ki67^{hi}$.

Methods