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 assumed that FM cells circulate freely in the lymphatic system, and so we pooled the numbers of these cells recovered from spleen and lymph nodes when modelling their dynamics. We compared different models of the population dynamics and structure of the FM compartment, as well as the possibilities that the T1 or T2 transitional subsets may serve as their source population. In order to compare the time-varying chimerism in FM cells across animals with different levels of bone marrow chimerism and different sources, we normalised the FM chimerism to that in the common progenitor population, *i.e.*T1 cells. Here, we assess the suitability of T1, T2 and pooled (T1+T2) compartments as putative source populations for FM cells and explore various mechanisms that can explain their population dynamics in mice.

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 chimerism within the FM compartment stablised at the level of the upstream T1 precursors (i.e. normalised donor fraction $f_d \to 1$) by about 210d 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. Additionally, we measured the kinetics of their expression of Ki67, a nuclear protein expressed during cell cycle and lost with a lifetime of a few 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$^{\text{hi}}$, but this proportion falls slowly to equalise with that of host cells at around 10% after approximately 100 days. This kinetic is consistent with IDEA HERE

We modelled this behaviour by assuming that cells differentiate into FM B cells at a rate proportional to the their source population, 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 also considered the possibilities that the direct precursors of FM B cells are T1 cells, T2 cells, or T1 and T2 combined. We fitted each model simultaneously to the timecourses of the total size, normalised donor chimerism normalised to T1 (the earliest common precursor to all populations considered) and the proportions of host and donor FM cells expressing Ki67 (Figure 1A and B; see Methods for details). We found strongest support for T1 as the direct precursor of FM B cells, and the 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 the source, whose kinetic was described empirically by fitting an empirical function to the timecourse of cell numbers. The data indicated the the size of the T1 and T2 populations changed very little with host age and so these functions were nearly flat. Time is measured from age 40 days, at which time the loss rate is δ_0. This model of time-dependent loss was superior to the simplest model with constant rates of division and turnover (ΔLOO-ic = 8) and also superior to the alternative with a time-varying division rate (ΔLOO-ic = 10). We estimate that FM B cells divide slowly, on average every 15 months, and have a mean residence time of $\sim$34 days in 7 week-old mice. This life expectancy doubles on an average every $\sim$28 months. We also predict that approximately 4% of FM B cells are replaced each day by newly differentiated cells from the T1 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. This suggests that in old animals individual FM clones and their progeny would persist longer in follicles than in younger animals, purely due to gradual increase in their survival.

No evidence for heterogeneity within FM B cells

The decline we detect in λ with host age, 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 T1 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 (δ_1 and δ_2) and division rates (ρ_1 and ρ_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 (ΔLOO-ic = 7, Table 1). Indeed there was a very weak signature of kinetic heterogeneity; the estimated loss and division rates of the two FM subpopulations were nearly equal (δ_1 = 0.38 (0.02, 1.1), δ_2 = 0.21 (0.01, 0.99), ρ_1 = 0.10 (0.001, 0.72), ρ_2 = 0.18 (0.0002, 0.63)).

We also found no evidence for any host-donor differences in kinetics in the form of a persistent host-derived 'incumbent' population (Table 1). For the discussion of the Incumbent model, see methods [Hogan et al. PNAS 2015 Rane et al. PLoS Biology 2018]. 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 (ΔLOO-ic = 18), hence rejected the possibility.

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.

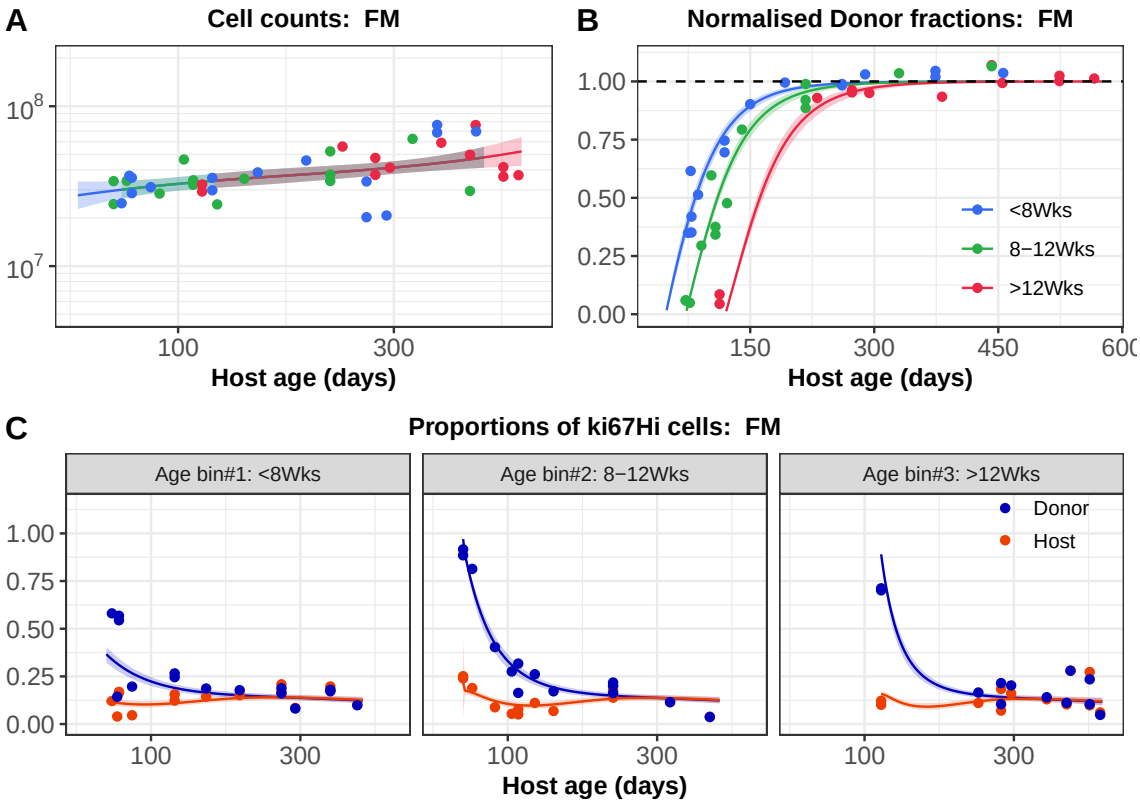

Figure 1: **Fitted and predicted population dynamics of FM B cells, using the best-fitting model in which cells divide at a constant rate and their mean residence time increases with host age.** The model was fitted simultaneously to the extended timecourses of total cell counts of FM B cells pooled from LN and spleen in busulfan chimeras, the donor fractions in FM B cells normalised to the chimerism in T1 cells and the proportion of cells that were $Ki67^{hi}$ within host and donor FM B cells. Solid lines denote the most probable description of the observations of (A) cell counts, (B) normalised donor fractions and (C) $Ki67^{hi}$fractions using the time-dependent model with envelopes indicating uncertainty in the model fit. Prediction intervals (4.5^{th} and 95.5^{th} percentiles) were plotted by drawing samples from the posterior distribution of parameter estimates. Different colours in (A) and (B) indicate the different groups of hosts, binned according to the age at which they were transplanted with donor BM cells. Model predictions for individual groups were drawn using the mean age at BMT.

	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 population dynamics of Follicular Mature (FM) B cells, pooled from LN and spleen.** 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)

.

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 of loss of Ki67 expression (days)	6.0	(4.6, 7.3)

Table 2: **Parameter estimates from the best-fit (Time-dependent) model using T1 compartment as the source population for FM B cells (Spleen + LN).** *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.

Germinal Center B cells

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

To explain the replacement kinetics of GC cells in busulfan chimeric mice, we used diverse models that address the heterogeneity in GC compartment and test the effects of host-age on their dynamics (described above in FM section and in Methods). All models were fitted simultaneously to an extensive time-course of total counts, donor fractions (f_d) and the proportions of Ki67^{hi}cells that spans over a year. The donor fractions in Spleen and LN GC compartments were normalised to the donor fractions in the common progenitor population, *i.e.*T1 cells, so as to compare them across animals with different levels of bone marrow chimerism. This also allows us to explore the suitability of splenic T1 and the pools of T2 and FM cells that circulate freely between spleen and LN as the putative source populations for spleen and LN GC cells.

Our data shows that the normalised f_d in splenic GC cells stabilises twice as fast as their lymph node counterparts ($\sim$110 and $\sim$260 days post BMT for spleen and LN GCs, respectively), suggesting a strong disparity in the invasion kinetics of donor cells between these two pools. 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?). Due to such differences observed in the population dynamics of spleen and LN GC cells, we decided to model them 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.

		Model and ΔLoo-ic			
Population	Source	Simple homogeneous	Time-dependent	Kinetic heterogeneity	Incumbent
SPGC	T1	17	9	33	12
	T2	10	0	33	9
	FM	13	8	32	9
LNGC	T1	55	41	12	54
	T2	50	42	12	51
	FM	12	35	0	38

Table 3: **Comparison of models describing population dynamics of GC B cells**. Loo-ic values obtained using leave-one-out cross validation method are shown relative to that of the best fitting model. We consider ΔLoo-ic values $\geq$6 of statistical significance for model selection.

The population dynamics of spleen GC cells are influenced by the age of the host.

We find that the total numbers of spleen GC cells increase over time but the proportions of Ki67^{hi}cells remain constant and almost identical between donor and host cells (Figure 2). These results suggest that there is either a substantial increase is the size of the source population or a decline in the turnover of spleen GC cells with host-age. As observed earlier, the size of FM subset increases over time, but their chimerism changes more slowly and stabilises much later (at $\sim$210 days post BMT) than in spleen GCs, suggesting that transitional (T1 and/or T2) subsets are more likely candidates for the source population. Indeed, we found that the model of time-varying loss (where the net loss rate λ declines with host-age) using T2 as the source of spleen GCs,

received strongest statistical support (Table 3) and produced best visual descriptions of the trends in total cell counts, normalised f_d and proportions of Ki67^{hi}cells in splenic GC cells (Figure 2).

Our analysis shows that rapidly dying spleen GC clones are sustained for about 15 days by equally rapid division rate in 7w old mice (Table 4). The ability of B cell clones to persist in splenic GC pool increases as animals get older due to a relative increase in their residence time $(1/\delta)$ in spleen, which doubles every $\sim$8 months. Models in which the rate of source influx or the inter-division time of GC cells varies with host-age produced poorer fits and received inferior statistical support (ΔLoo-ic ≥ 8). We also find that the maintenance of spleen GC compartment is mainly dependent on the source influx as a fairly large proportion of GC cells in spleen ($\sim$ 36% of total counts in 7w old mice) are daily replaced by the cells from the circulatory pool of T2 compartment.

Parameter	**Estimates and 95% CI**
Source influx ($\#$of cells $\times 10^{-3}$)	4.7 (3.2, 6.8)
Mean clonal lifespan 'τ' at 7w of host age (days)	15 (13, 16)
Mean resident time at 7w of host age (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 of loss of Ki67 expression (days)	5.2 (3.8, 6.7)

Table 4: **Parameter estimates from the best-fit (Time-dependent) model using T2 compartment as the source population for SP GC B cells.** 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.

Lymph node GC population contains the mixture of transient and persistent clones.

The GC compartment in lymph nodes shows a modest increase in its size over time with steady and high proportions of Ki67^{hi}cells that are almost identical between host and donor sub-populations (Figure 3C), very similar to what observed in the spleen. We found that the models with assumptions of homogeneity within the GC compartment failed to capture the trend in total cell numbers and slow stabilisation of the normalised f_d, even after allowing host-age dependent alterations in their turnover. The replacement kinetics of LN GC cells were best explained by the model that assumes kinetically distinct subsets within GC population, as reflected by its visual descriptions of the trends in cell counts, normalised f_d and fractions of Ki67^{hi}cells in LN GC compartment (Figure 3) and superior statistical support (Table 3). Our data also identified FM compartment as the most likely source of LN GCs as compared to T1 and T2 Transitional subsets (ΔLoo-ic $\geq$ 12, Table 3).

Our analysis reveals that some GC clones are extremely short-lived and are cleared from the lymph nodes within days (average clonal lifespan of $\sim$ 5days, Table 5) while others can persist for as long as 2 months (Table 5). The presence of pre-existing host-derived 'persistent' GC clones may explain the slow replacement kinetics of GC compartment in lymph nodes. In a 7 week old mouse, the LN GC compartment is equally divided between 'transient' and persistent clones and we speculate that this ratio would skew towards latter in older animals. The accumulation of persistent clones over time may also explain the gradual increases in the pool size of LN GC cells. We also find that roughly 25% LN GC cells are replaced daily by the new cells entering from the pool of FM cells that are freely circulating between spleen and LN.

Parameter	**Estimates and 95% CI**	
	Transient Subset	Persistent Subset
Source influx ($\#$of cells $\times 10^{-2}$)	7.0 (0.2, 40)	24 (12, 43)
Mean clonal lifespan (days)	5.0 (1.2, 19)	68 (34, 130)
mean resident time (days)	0.69 (0.42, 1.3)	0.90 (0.46, 1.9)
mean inter-division time (days)	0.91 (0.47, 2.0)	0.79 (0.46, 1.9)
Fraction of total population at 7w of host age	0.51 (0.17, 0.72)	0.49 (0.28, 0.83)
Average time of loss of Ki67 expression (days)	5.1 (3.7, 6.6)	5.1 (3.7, 6.6)

Table 5: **Parameter estimates from the best-fit (Kinetic heterogeneity) model using FM compartment as the source population for LN GC B cells.** 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$^{\text{hi}}$cells while GC are $\sim$ 95% Ki67$^{\text{hi}}$.

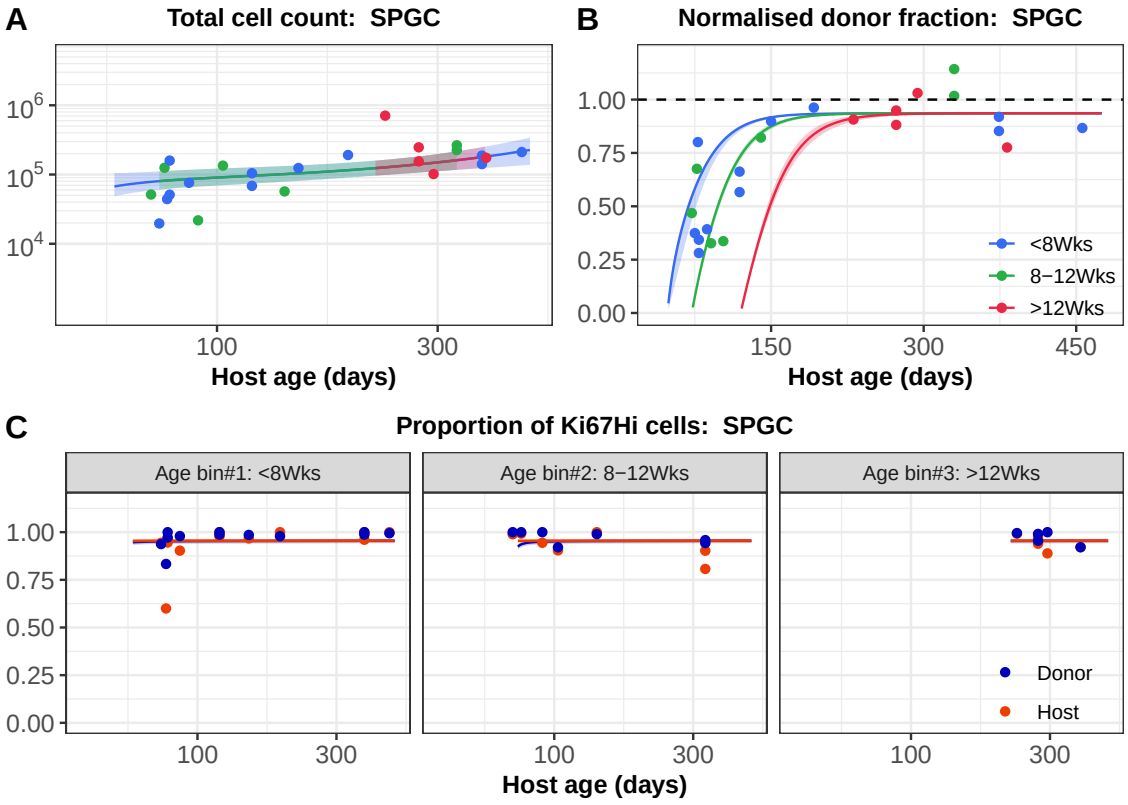

Figure 2: **Fitted and predicted population dynamics of spleen GC B cells, using the best-fitting Time-dependent model with T2 as the source.** The model was fitted simultaneously to the time-course of total cell counts of spleen GC B cells, the donor fractions normalised to the chimerism in T1 cells and the proportions of $Ki67^{hi}$cells within their host and donor compartments. The time-dependent model fit to the time-course of normalised f_d stabilises at a value <1, precisely equal to the ratio of donor fraction in T2 to the donor fraction in T1. Solid lines denote the most probable description of the observations of (A) cell counts, (B) normalised donor fractions and (C) $Ki67^{hi}$fractions using the time-dependent model with envelopes indicating uncertainty in the model fit. Prediction intervals (4.5^{th} and 95.5^{th} percentiles) were plotted by drawing samples from the posterior distribution of parameter estimates. Different colours in (A) and (B) indicate the different groups of hosts, binned according to the age at which they were transplanted with donor BM cells. Model predictions for individual groups were drawn using the mean age at BMT.

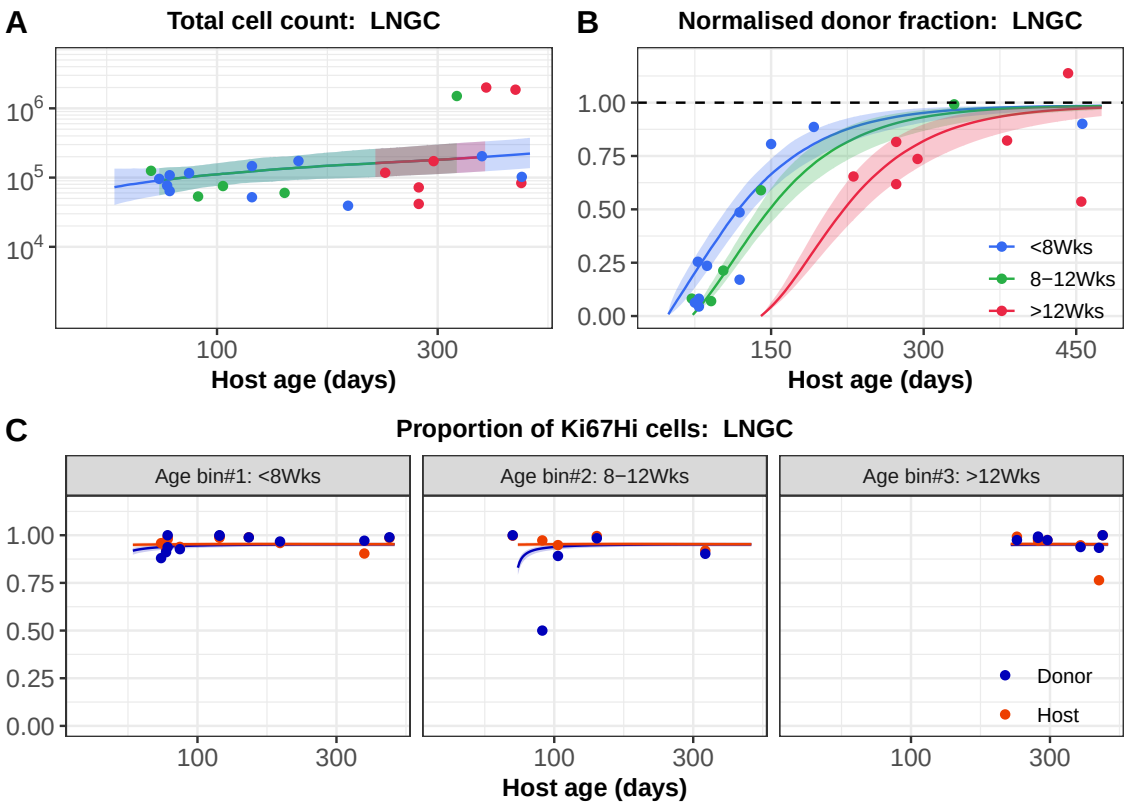

Figure 3: **Fitted and predicted population dynamics of LN GC B cells, using the best-fitting Kinetic heterogeneity model with FM as the source.** The model was fitted simultaneously to the time-course of total cell counts of GC B cells pooled from multiple lymph nodes of busulfan chimeric mice, the donor fractions normalised to the chimerism in T1 cells and the proportions of $Ki67^{hi}$cells within their host and donor compartments. The kinetic heterogeneity model fit to the time-course of normalised f_d approaches 1, following the trend in donor fraction in FM normalised to the donor fraction in T1. Solid lines denote the most probable description of the observations of (A) cell counts, (B) normalised donor fractions and (C) $Ki67^{hi}$fractions using the time-dependent model with envelopes indicating uncertainty in the model fit. Prediction intervals (4.5^{th} and 95.5^{th} percentiles) were plotted by drawing samples from the posterior distribution of parameter estimates. Different colours in (A) and (B) indicate the different groups of hosts, binned according to the age at which they were transplanted with donor BM cells. Model predictions for individual groups were drawn using the mean age at BMT.