Research A

transfer system (Phan et al, 2005). SW$_{HEL}$ transgenic B cells bear a rearranged hen egg lysozyme (HEL)–specific VDJ$_H$ element targeted into the IgH chain locus combined with an HEL-specific k L-chain transgene (Phan et al, 2005). CD45.2$^+$ - $^{+\ +}$ or $^{-\ -}$ SW$_{HEL}$ B cells were adoptively transferred into wild-type CD45.1$^+$ congenic recipients and immunized with HEL coupled to sheep red blood cells (HEL-SRBCs—Fig 1A) to promote a T-dependent response.

We started by measuring the effects of miR-155 on the kinetics of the B-cell response. In the SW$_{HEL}$ system, B-cell blasts can be detected in the periarteriolar lymphoid sheath as early as 1 d after HEL-SRBC immunization and commence proliferation from 1.5 d (Chan et al, 2009), and plasmablasts can be detected at day 3.5, they peak by day 4.5 and rapidly decline afterward (Paus et al, 2006; Phan et al, 2005). Adoptively transferred miR-155–sufficient or miR-155–deficient splenic B cells were stained for HEL B-cell receptor (BCR) in combination with CD45.1, CD45.2, CD138, FAS, and B220 andor(0)-111Tf3.3

also shown to be virtually all FAS$^+$ (Fig 1B). In the absence of miR-155, also analyzed using GOrilla. Some of the up-regulated processes involved regulation of cellular metabolic processes, mRNA splicing, as well as histone and chromatin modification (Fig 4D and Table S2).

The results in this report demonstrate a critical in vivo role for miR-155 in proliferation and survival of plasmablast B cells in response to the T-cell–dependent antigen HEL. We have used the SW_{HEL}

2002). Previously, most of the roles of miR-155 in B-cell differentiation have been attributed to regulation of the germinal centre response, whereas the requirement for miR-155 in the extrafollicular plasmablast response has not been well characterised. Our data are significant in elucidating miR-155 as a new player in the early expansion of antigen-specific B-cell blasts into extrafollicular plasmablasts, which is necessary for short-term immune protection to infection (Nutt et al, 2015). The onset of the proliferation defect in $^{-/-}$ B-cell blasts occurs at the stage of B:T cell interaction, and we also previously showed that dysregulation of PU.1 by miR-155 in cultured B cells affects the expression of genes involved in adhesion and B:T cell interaction (Lu et al, 2014). However, SW$_{HEL}$ $^{-/-}$ mice from day 3.5 post HEL-SRBC immunisation are still able to form germinal centres or locate in the red pulp (Nakagawa et al, 2016), suggesting that loss of cellularity in the absence of miR-155 is not explained by impaired migration and that, to some extent, miR-155–deficient B-cell blasts are responsive to T-cell help. The resemblance of our phenotype to that observed in IL-21 or IL-21R receptor–deficient mice (Lee et al, 2011), however, may indicate a potentially impaired response to T-cell help. In the absence of IL-21R, both the extrafollicular plasmablasts and the germinal centre responses are impaired to 10% of wild-type

–defiaffien.anrmore cn(n m ed)4465.9on86.952cen.po therefore, be interesting to investigate further whether the defects observed with miR-155 deficiency are linked to defective IL-21 signalling.

CD45.1$^+$ congenic mice were bred and maintained in the Babraham Research Campus small animal facility. SW$_{HEL}$ mice and miR-155–deficient mice and have been described previously (Phan et al, 2003; Rodriguez et al, 2007). SW$_{HEL}$ mice were a gift from R. Brink (Garvan Institute of Medical Research/University of New South Wales). All mice were on the C57BL/6 background and bred and maintained in the Biological Support Unit of Babraham Institute under specific opportunistic pathogen-free conditions.

A

SW$_{HEL}$ $^{+/+}$ or SW$_{HEL}$ $^{-/-}$ donor B cells were adoptively transferred into nonirradiated CD45.1$^+$ congenic recipient mice followed by injection of 2 10^8 HEL-SRBCs (Fig 1A). HEL (Sigma-Aldrich) was conjugated to SRBCs, and expression was subsequently measured with anti-HEL HyHEL9 antibody by flow cytometry. L1.6(Tf0.575758Ho.4(anti-4liro 9TD(44bo9-1.3.7andmea -65945(er)-3

Splenocytes were enriched by CD45.1-negative selection using an autoMACS pro separator (Miltenyi Biotec).

[illegible]

Multicolor flow cytometry for analysis or for sorting was performed on an LSR Fortessa-5 or FACS Aria (BD Biosciences), respectively. Single-cell suspensions of splenocytes were blocked with anti-CD16/32 mAb (clone 2.4G2), followed by staining with the following antibodies: anti-B220 (clone RA3-6B2) and anti-CD45.2 (clone 104) from BD Biosciences. HEL-binding B cells were stained as described previously (Chan et al, 2009). For cell cycle analyses, spleen cells were first stained for extracellular antigens and then were analysed with 10 μg/ml DAPI staining using a Cytofix/Cytoperm kit (BD Biosciences) or PFA and Tween-20. Cell cycle was calculated by FlowJo Dean/Jett/Fox algorithm or by setting gates manually. The Click-iT EdU Alexa Fluor 488 Imaging kit and CaspGLOW Fluorescein Active Caspase Staining kit (both from Thermo Fisher Scientific) were used according to the manufacturers' instructions. Data were analysed with FlowJo software (Tree Star).

[illegible]

After sorting of plasmablast B cells directly into Trizol, RNA was extracted and resuspended in RNase-free water. RNA that passed quality control using a bioanalyser and NanoDrop was subject to rounds of amplification using the Ambion Expression kit. cDNA from five independent biological samples of SW$_{HEL}$ $^{+/+}$ or SW$_{HEL}$ $^{-/-}$ sorted plasmablast B cells were hybridized to GeneChip Mouse Gene ST1.0 arrays (Affymetrix) according to the manufacturers' instructions. Bioconductor package affy and the robust multiarray average function were used for background correction, and normalization was performed using the software package R by the Babraham Bioinformatics facility. Normalized data were filtered with a threshold of the modal expression value in which three of the five samples had to exceed the log2 modal expression threshold. Differentially expressed genes between miR-155–sufficient or miR-155–deficient plasmablast B cells were assessed using a P-value less than 0.05 and a fold change of greater than 1.3-fold.

[illegible]

Differentially expressed genes in miR-155–deficient plasmablast samples were computed using the GOrilla tool (Eden et al, 2009) to determine enriched gene ontology terms. A background list of genes was included in the analysis. If several related terms were significantly enriched, the terms with a higher percentage of differentially expressed genes were chosen, and are presented in Fig 4.

[illegible]

Total RNA was extracted from sorted plasmablast populations using TRIzol (LifeTech). RNA from sorted plasmablast B cells was converted to cDNA according to the SuperScript reverse transcriptase III protocol (Invitrogen) and then analysed by RT-qPCR. Cell cycle genes E2F1, E2F2, Myc, and Myb were analysed using custom or commercially available primers (see Table S3). E2F1 and E2F2 mRNA transcript expression was analysed using primers according to Pilon et al (2008). Myc, E2F1, and E2F2 RT-qPCR assays were analysed using Platinum SYBR Green qPCR SuperMix (Life Technologies). Relative abundance was calculated using a standard curve or δCT method and normalized to the expression of mRNA-encoding HPRT. Myb RT-qPCR assays were performed with Taqman assays. Expression of Myb mRNA was calculated using a standard curve and normalized to the expression of β2M.

[illegible]

Statistical analyses were performed in GraphPad Prism software or R Studio; the tests are indicated in the figure legends. All data were tested for normality of residuals. If data were normally distributed, parametric tests were used. For non-normally distributed data, several transformations were attempted, and if this was satisfactory, parametric tests were used. Where transformation of data yielded non-normally distributed residuals, nonparametric tests were used. For B-cell blast number, data were square-root-transformed. For testing the effects of genotype on plasmablast and germinal centre cell number, the data were log-transformed and tested by two-way ANOVA. There was a significant interaction between genotype and time. For CFSE data, the values were arcsine-transformed. For EdU incorporation data, the values were arcsine-transformed and tested by two-way ANOVA.

[illegible]

All mouse experiments were approved by the Animal Welfare and Ethical Review Body of the Babraham Institute. Animal husbandry and experimentation complied with the existing European Union, United Kingdom Home Office legislation and local standards.

[illegible]

Supplementary Information is available at https://doi.org/10.26508/lsa.201800244.

[illegible]

We wish to thank the Biological Services Unit, Flow Cytometry core, and Bioinformatics facilities of the Babraham Institute for expert technical assistance; Dr Robert -497.5(For)-489.64a,and local standards.

A2 2

G Arbore: data curation, formal analysis, investigation, and methodology.
T Henle: data curation, formal analysis, investigation, and methodology.
L Biggin: formal analysis and investigation.
S Andre: formal analysis and investigation.