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Identification of a new subset of lymph node stromal cells involved in regulating plasma cell homeostasis

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Abstract

Antibody-secreting plasma cells (PC) arise rapidly during adaptive immunity to control infections. The early PC are retained within the reactive lymphoid organ where their localization and homeostasis relies on extrinsic factors, presumably produced by local niche cells. While myeloid cells have been proposed to form those niches, the contribution by co-localizing stromal cells has remained unclear. Here, we characterized a subset of fibroblastic reticular cells (FRC) that forms a dense meshwork throughout medullary cords of lymph nodes (LN) where PC reside. This medullary FRC type is shown to be anatomically, phenotypically and functionally distinct from T zone FRC, both in mice and humans. By using static and dynamic imaging approaches, we provide evidence that medullary FRC are the main cell type in contact with PC guiding them in their migration. Medullary FRC also represent a major local source of the PC survival factors IL-6, BAFF and CXCL12, besides producing also APRIL. *In vitro*, medullary FRC alone or in combination with macrophages promote PC survival while other LN cell types do not have this property. Thus, we propose that this new FRC subset, together with medullary macrophages, forms PC survival niches within the LN medulla, and thereby helps promoting the rapid development of humoral immunity which is critical in limiting early pathogen spread.

Significance statement:

Lymph nodes are sites where adaptive immunity is initiated, leading to the generation of plasma cells secreting large amounts of antibodies that typically interfere with pathogen spread. Plasma cells are known to depend on extrinsic factors provided by niche cells to stay alive; however, the critical niche cells are still poorly understood. Here we present evidence for a new fibroblast subset within murine and human lymph nodes that is unique to the medulla where plasma cells reside. These fibroblasts produce factors that positively regulate plasma cell homeostasis, similar to macrophages. Knowing the critical niche cells may help to design intervention strategies to target this niche in the setting of autoimmune disease caused by plasma cells secreting autoreactive antibodies.

“/body”

INTRODUCTION

Lymph nodes (LN) are specialized organs filtering lymph fluid and inducing adaptive immunity to antigens derived from pathogens, tumors or vaccines. They are composed of different regions, each populated by distinct resident stromal cells, including follicular dendritic cells (FDC), that attract recirculating B cells into follicles, and fibroblastic reticular cells (FRC), that attract recirculating T cells and mature dendritic cells (DC) into the paracortex (1, 2). The third compartment - the medulla - is a site of plasma cell (PC) residence and antibody secretion. So far, the stromal organizer cell of that zone has been poorly characterized.

PC responses are crucial for systemic antibody-mediated protection upon infection, and are the main success reason of current vaccines. Upon B cell activation within LN, two anatomical niches critical for an efficient humoral response develop: PC foci within medullary cords (MC) and germinal centers (GC) within follicles (3, 4). The extrafollicular B cell differentiation within MC allows rapid antibody responses to limit systemic spread of replicating pathogens. Typically, within 2-4 days after infection, proliferating plasmablasts (PB) are observed in outer T zones which differentiate into PC that take residence within MC and secrete large quantities of antibodies. This early humoral response of lower affinity usually lasts few days with only a small fraction of PC surviving several weeks (3, 4). In contrast, the follicular B cell differentiation within GC allows the generation of memory B cells and PC of high affinity, with PC exiting the LN and homing to sites like the bone marrow (BM), where they can survive over months and provide long-term humoral protection starting around day 10-14 after immunization (4, 5).

PC isolated from BM, LN and spleen are mostly short-lived when placed *in vitro* (6, 7), raising the question about the critical support cells providing extrinsic survival signals *in vivo*. The prevailing view is that myeloid cells, including macrophages, DC and

granulocytes, mediate PC survival within activated LN and spleen (4, 8). This is based both on evidence of co-localization in PC-rich areas and expression of factors which can increase PC survival *in vitro*, including BAFF, APRIL, IL-6 and CXCL12 (4, 6, 8). However, myeloid cells were found to be a minor source of BAFF and CXCL12 *in vivo* (8), consistent with an important role for non-hematopoietic cells as BAFF source in the extrafollicular PC response (9). In addition, *in vivo* cell depletion experiments showed that myeloid cells have either a positive role (10), no role (11) or even a negative role in extrafollicular PC development (12).

In the BM, fibroblasts form part of the survival niche for long-lived PC by producing IL-6 and CXCL12, along with hematopoietic cell types contributing BAFF and APRIL (4). Poorly characterized fibroblasts from LN and spleen in mice and men were also shown to promote PC survival *in vitro* (7, 13-15), raising the possibility that FRC-like cells within MC may contribute to PC homeostasis.

FRC expressing podoplanin (pdpn, also known as gp38) are found in all three peripheral LN (pLN) compartments, produce extracellular matrix (ECM) proteins and are heterogeneous in their localization, morphology and function (1, 16). T zone FRC (TRC) are the largest and best studied subset, forming a 3-dimensional cell network which physically guides lymphocyte migration (17). They support T cell function through the secretion of the survival factor IL-7 and the chemokines CCL19 and CCL21, responsible for T cell and DC attraction, motility and retention within T zones (2, 16). TRC wrap around a matrix-based tube system, called conduits, used for intranodal transport of lymph fluid and small molecules (18). Within follicles two additional FRC subsets were described that express the naïve B cell survival factor BAFF and the B cell attractant CXCL13: MAdCAM^{+} marginal reticular cells (MRC) localizing in the subcapsular region (19), and MAdCAM^{-} FRC in the outer follicle (20, 21). FRC depletion experiments have shown that lymphocyte responses cannot be induced efficiently in the absence of FRC (22). Together, these data demonstrate

that distinct FRC types act as organizer cells of microenvironments found within the B and T zone, and thereby play a critical role in adaptive immunity.

Given that matrix fibers and pdpn^{+} FRC-like cells have been observed by histology within MC by us and others (10, 18, 23-25), we hypothesized that a specialized FRC subset may serve as the missing positive regulator of local PC niches. Here, we have performed a detailed histological and functional analysis of FRC subsets within activated LN. We report the identification of medullary FRC (MedRC) along with the characterization of their unique morphological, molecular and functional properties. Importantly, using histological and functional assays we demonstrate that MedRC are a major structural component of the PC niche in the medulla guiding PC migration and residence, but also a potent source of PC attraction and survival factors.

RESULTS

Histological identification of medullary FRC in murine and human LN

To generate a strong PC accumulation within LN, mice were immunized s.c. with ovalbumin (OVA) protein in Montanide (Mont) adjuvant. 5-8 days later strongly enlarged medullary areas were observed, composed of LYVE1$^+$ medullary sinuses (MS) and MC enriched in CD138$^+$ PC, but only few scattered B and T cells (Fig. 1A-B). Networks of pdpn$^+$ desmin$^+$ FRC and laminin$^+$ matrix structures were not only observed throughout T zones but also within MC, often organizing around CD31$^+$ HEV (Fig. 1B-C; SI Appendix Fig. S1A). Similar to TRC, reticular cells of the MC express desmin, αSMA and LTβ-receptor (SI Appendix Fig. S1B). CD11b$^+$ or CD169$^+$ macrophages were predominantly seen within MS, with some being inside MC (Fig. 1C). When screening various stromal cell antibodies we noted that BP3 (CD157) labels both MRC and TRC, but not MedRC (Fig. 1D; SI Appendix Fig. S1C), while MAdCAM-1 marks only MRC in naïve and activated lymph nodes. Thus, the combination of MAdCAM-1 and BP3 allows the distinction of MedRC from TRC and MRC in murine LN. CD34, however, marks only a subset of MedRC found close to medullary blood vessels (SI Appendix Fig. S1D).

Next, we assessed whether in human reactive LN sections these stromal cell markers would identify a similar MedRC subset colocalizing with PC. Medullary regions were readily observed, displaying MC densely populated by nucleated cells and centered around CD31$^+$ blood vessels while being surrounded by LYVE1$^+$ MS harboring few cells in the lumen (Fig. 1E). CD138$^+$ PC localize preferentially to the MC, clearly separated from most T and B cells. As expected (26), pdpn shows the strongest expression on TRC, which were CD21$^-$, while FDC co-express pdpn and CD21 (Fig. 1F). Within MC, pdpn labels reticular cells, as well as vascular cells expressing CD31 and/or LYVE1 (Fig. 1E). In contrast to the mouse, BP3 expression is highest in the medulla, intermediate on TRC and low on FDC (Fig. 1F; SI Appendix Fig. S1E). Within MC, BP3 expression was observed on a dense reticular cell

network that partially co-localizes with pdpn$^+$ cells. Consistent with them being fibroblastic cells, MC regions display collagen-I and laminin-expressing reticular fibers. They are part of the PC niche (Fig. 1G), as are CD11c$^+$ DC and lysozyme$^+$ macrophages/granulocytes (SI Appendix Fig. S1F). Thus, the PC niche composition in human LN medulla closely resembles the one observed in mice.

Identification of medullary FRC by flow cytometry

Next, the surface markers MAdCAM-1 and BP3 were tested by flow cytometry on CD45$^-$35$^-$31$^-$pdpn$^+$ FRC isolated from murine LN. Three FRC subsets were observed in both naive and activated LN, with MAdCAM$^+$BP3$^+$ MRC representing 5-10%, MAdCAM$^-$BP3$^+$ TRC representing 50-65%, and MAdCAM$^-$BP3$^-$ MedRC 20-35% of isolated FRC (Fig. 2A). During immune response only MRC slightly increased in proportion, with all subsets increasing 4-fold in number and 40-80% of cells having incorporated BrdU (Fig. 2B-C; SI Appendix Fig. S2A-B).

Given the distinct immune cell composition in MC we reasoned that MedRC should be functionally different from CCL21$^+$ TRC and CXCL13$^+$ MRC. Indeed, MedRC from both naive and activated LN showed very little intracellular CCL21 protein, while many TRC from wild type (wt), but not CCL19/21ser-deficient *plt/plt* mice, were CCL21$^+$ (Fig. 2D; SI Appendix Fig. S2C). While some MRC-like cells express CCL21, other MRC showed higher CXCL13 expression than TRC, with MedRC being CXCL13$^-$. This chemokine expression pattern was largely maintained during the immune response (SI Appendix Fig. S2D). These findings were confirmed in sorted cells at the transcriptional level. *ccl21* and *cxcl13* mRNA were highest in TRC and MRC of naive and activated LN, as were *ccl19* and *il7* (Fig. 2E; SI Appendix Fig. S2E). Importantly, MedRC had at least 10-fold lower levels of these four cytokine transcripts. In contrast, *cxcl12* was expressed mainly by MedRC and TRC. Sorted lymphatic and blood endothelial cells (LEC/BEC), however, did not show much

expression for any of these cytokines. These qRT-PCR data corroborate with *in situ* hybridization (ISH) analysis, showing the very modest expression levels of *ccl21*, *cxcl13* and *ccl19* transcripts within MC, while *cxcl12* transcripts were found to be at levels comparable to the T zone (Fig. 2F; SI Appendix Fig. S2F). Histological staining for chemokine proteins confirmed the lack of CCL21 and CXCL13 within MC, while CXCL12 was readily detected, both in MC and T zones, and frequently co-localizing with reticular pdpn^{+} FRC (Fig. 2G). Thus, our data demonstrate that MedRC are anatomically, phenotypically and functionally distinct from TRC and MRC. Multiparameter analysis by flow cytometry confirm this notion, and indicate an even greater diversity of pdpn+ fibroblastic LN cells than previously anticipated, with a continuum of 7 phenotypically different FRC populations (Fig. 2H and S2G), which do not include FDC and pdpn-CD31- populations.

MedRC and TRC generate different extracellular matrix (ECM) structures

TRC produce functional conduits that they enwrap but it is not known whether similar features apply to MedRC. We observed a reticular staining for collagen I and IV as well as fibronectin, often in thinner structures than inside T zones but often co-localizing with pdpn^{+} MedRC (Fig. 3A). Consistent with these findings, all three FRC subsets express high levels of *collagen1α1* and *collagen1α2* transcripts, with a marked increase after immunization, especially in MedRC (Fig. 3B). Given the high specificity of collagen 1 expression for fibroblasts, we used transgenic mice expressing GFP under control of the collagen 1α1 promoter (pCol-GFP) (27) to visualize FRC *in vivo*. In activated LN, a reticular GFP expression was clearly visible, being strongest in MC, intermediate inside T zones and absent in the center of follicles (Fig. 3C). By histology and flow cytometry GFP expression is largely restricted to the three FRC subsets (SI Appendix Fig. S3A-D). Similar to GFP expression, histological PDGFRα staining was stronger on MedRC than TRC, while

BP3 and CCL21 showed the opposite pattern (Fig. 3D; SI Appendix Fig. S3D-G). This segregation was reflected in the lymphocyte distribution, with T cells co-localizing with CCL21$^+$ TRC and scattered B cells found among MedRC (SI Appendix Fig. S3D, F).

The increased GFP signal detected by fluorescence microscopy in MC relative to T zones correlated with a 2-fold higher FRC density and a therefore reduced spacing between FRC processes (Fig. 3E; SI Appendix Fig. S3E). In addition, MedRC display less elongated cell bodies and thinner cell processes than TRC (Fig. 3E). Similar to the collagen I staining, second harmonic generation (SHG) revealed differences in the fibrillar collagen organization. In the medulla, thick and wavy collagen bundles alternate with very thin fibers, while in T zones collagen fibers display a more linear and regular organization (Fig. 3 F-G; SI Appendix Movie S1). Interestingly, MedRC enwrapped less the matrix structures than did TRC (Fig. 3G). To test conduit function, fluorescent dextran was injected subcutaneously. While most tracer localized to LYVE1$^+$ MS and to T zone conduits, a thin reticular pattern was apparent in MC, overlapping with collagen IV staining (Fig. 3H; SI Appendix Fig. S3H). In conclusion, MedRC build a very dense cell network that is only partially associated with an equally dense but functional conduit network.

Medullary FRC show extensive contacts with PC

Next, we compared the relative contribution of hematopoietic cells versus MedRC to the PC niche. Numerous macrophages (F4/80$^+$, CD11b$^+$ or CD169$^+$), DC (CD11c$^+$, MHCII$^+$) and B220$^+$ B cells are present in MC and show direct PC contact, while the frequency of granulocytes (Gr1^{hi}) is much lower (Fig. 4A; SI Appendix Fig. S4A). MedRC were observed in MC in a similar density as macrophages (SI Appendix Fig. S4B). While 60-70% of PC physically interacted with macrophages and B cells, almost 100% of PC contacted GFP$^+$ MedRC (Fig. 4A). The latter finding was

confirmed and extended using 3D confocal image stacks, where on average 25% of the PC surface was found to physically touch MedRC, often at multiple points (SI Appendix Fig. S4C; SI Appendix Movie S2). Despite forming a dense network in MC, MedRC do not contact all cells equally, as only 70% of B220^{+} cells contact them, in contrast to 100% of PC and F4/80^{+} cells (SI Appendix Fig. S4D).

To gain insight into the dynamics of PC interactions with MedRC, labeled PC (CD138^{+}B220^{low}) or PB (CD138^{+}B220^{int}) were added on top of viable tissue slices of activated LN from pCol-GFP mice. Most PB migrated preferentially into the MC where endogenous CD138^{+} PC resided (SI Appendix Fig. S4E). As previously reported (28), PB showed higher motility than PC, more prominently within the outer T zone than the MC, and this migration was largely unaffected by pertussis toxin, which blocks most chemokine-based cell migration. Next, endogenous PC/PB within viable LN slices were antibody-labeled to image their dynamic interaction with GFP^{+} FRC. In the outer T zone, PB moved rapidly and often in long linear tracks, thereby following the TRC network, going from one TRC to the next, but with no directional bias toward MC (Fig. 4B-C and S4F; Movies S3-4). In contrast, PC in MC showed much slower and confined migration with limited displacement (Fig. 4B-C; SI Appendix Movie S5). Consequently, PC interactions with each MedRC were considerably longer, changes from one MedRC to the other less frequent, and contact always continuous. MedRC frequently showed pronounced morphological changes, in contrast to the more static TRC (SI Appendix Movie S6), possibly due to the difference in matrix association. Collectively, these data show that MedRC physically guide PC migration and residence, by providing both a cellular network and niche-like structures.

Medullary FRC are a rich source of plasma cell survival factors

To look for the potential expression of PC survival factors by MedRC, they were sorted along with seven other cell types. Strikingly, on d5 and d8 activated LN *baff* transcripts were 40-100fold more abundant in the three FRC subsets than in macrophages and DC, with rare granulocytes showing intermediate levels (Fig. 5A; SI Appendix Fig. S5A, B). In contrast, *april* transcript levels were similar among FRC subsets, macrophages and DC. *Il6* transcripts were 10-20fold higher in MedRC than in other FRC subsets and myeloid cells. Finally, *Cxcl12* was highly enriched in the three FRC subsets. Surprisingly, expression of these four cytokines were similar in cells sorted from activated versus naïve LN (SI Appendix Fig. S5B), pointing to an increase in the number of expressing cells rather than expression levels.

Given that we saw a preferential expression of BAFF and IL-6 transcripts in FRC, especially MedRC, we wished to localize them in sections of activated LN, similar to our previous analysis for CXCL12 (Fig. 2F, G). BAFF protein staining of activated wt LN was strongest within MC, with lower expression detectable within T and B zones. Part of the medullary BAFF colocalized with pdpn^{+} FRC. To identify the BAFF source more precisely, activated LN of mice transgenic for a non-cleavable BAFF were investigated. Strikingly, the BAFF staining was much stronger than in wt LN, with highest levels on pdpn^{+}/desmin^{+} MedRC and TRC (Fig.s 5B and S5C-D). IL-6 transcripts were also abundantly present within MC, besides their expression in B and T zones (Fig. 5C). To further define the cellular sources of IL-6 and BAFF at the protein level, various cell types were sorted, cultured and assessed by ELISA. *Ex vivo* MedRC proved to be the richest source of IL-6 protein when compared with TRC, macrophages and DC (Fig. 5D). In contrast, we failed to detect secreted or surface bound BAFF. Thus, our results suggest that within the PC niche MedRC are a major source of IL-6, BAFF and CXCL12, with hematopoietic cells contributing only APRIL and possibly IL-6 at comparable levels.

IL-6 is important for plasma cell differentiation or survival in peripheral LN

Given the predominant IL-6 expression by MedRC, we tested the *in vivo* role of IL-6 for the extrafollicular PC response. IL-6 competent splenocytes from OT-I and OT-II TCR tg mice were transferred into IL-6 deficient mice followed by OVA/Mont immunization, which allowed a normal T cell expansion (SI Appendix Fig. S6A). On d5.5 and d8.5 after immunization, PB/PC numbers in the draining LN were reduced by 60% in IL-6 KO mice relative to controls (Fig. 6A; SI Appendix Fig. S6A). This result cannot be explained by altered B cell development in IL-6 KO mice, because transferred wt B cells showed a similar reduction in local PB/PC differentiation as $IL6^{-/-}$ B cells. We noted a marked reduction in various stromal cells within activated LN, including MedRC but not TRC, suggesting that IL-6 directly or indirectly regulates the number of MC niches (SI Appendix Fig. S6B). OVA-specific IgM and IgG levels in the serum were approximately 2-fold reduced in IL-6 deficient mice (SI Appendix Fig. S6C). Strikingly, OVA-specific IgG produced in cultures from IL-6 deficient LN were almost 10-fold reduced at both time points investigated and correlated well with the rare occurrence of IgG^+ PB/PC, while the local IgM response was poorly affected (Fig. 6A-B). This striking scarcity of IgG^+ PC raised the possibility that PC in IL-6 KO mice have defects either in their migration or retention in MC, where they complete their maturation into PC, or in their survival. In $IL\text{-}6^{-/-}$ mice, the localization and clustering of $CD138^+$ PC next to MedRC was unaltered (SI Appendix Fig. S6D). Similarly, IL-6 neutralization had no effect on endogenous PC localization in MC and no or minor effects on their velocity and spatial displacement (Fig. 6C-D; SI Appendix Movie S7). Thus, IL-6 may primarily serve as factor driving the development and/or survival of PB/PC, including possibly a role in isotype switching.

Medullary FRC and macrophages promote PC survival via IL-6 production

Genetic tools to selectively manipulate MedRC *in vivo* are currently lacking. Therefore, we turned to *in vitro* co-culture assays to test whether MedRC and innate immune cells are capable of regulating PB/PC homeostasis. While all purified PB/PC died when cultured alone for 3 days, 10-20% of PB/PC were rescued in presence of FRC isolated from activated LN to an extent similar to recombinant IL-6 (Fig. 7A). This PB/PC rescue involved very little PB proliferation *in vitro* (SI Appendix Fig. S7A) indicating that this assay is mainly a readout for PC survival. PC numbers were preserved when PC were separated from FRC by a transwell filter, or when PC were cultured with FRC conditioned medium, suggesting the survival factor is soluble rather than membrane-bound (Fig. 7A). FRC-mediated PC survival was blocked almost entirely by IL-6 neutralizing antibodies but not by inhibitors of BAFF, APRIL or CXCL12 (SI Appendix Fig. S7B). However, a marked drop in *baff*, *april* and *cxcl12* expression was noted in cultured FRC, with minor effects for IL-6, which may be lost later on, as indicated by the low levels observed in the FRC line pLN2 (SI Appendix Fig. S7C). Next, the various LN cell types were FACS-sorted and tested in their capacity to mediate PC survival. MedRC and macrophages were much more efficient in mediating PC survival compared to sorted TRC and DC, with no PC survival observed in LEC/BEC or T/B lymphocyte co-cultures (Fig. 7B). PC survival by MedRC, macrophages or DC was almost exclusively mediated by IL-6, as was their capacity to boost IgM and, more impressively, IgG production (Fig. 7C, D). Combining the various cell types, each in suboptimal numbers, showed synergistic effects on PC survival and additive effects on IgG but not IgM secretion, with most of the effects mediated by IL-6 (Fig. 7E; SI Appendix Fig. S7D). Interestingly, MedRC seem to be more efficient in promoting IgM/G secretion by PC than macrophages or DC, also indicating that PC survival is not predictive of Ig secretion levels which may depend on high IL-6 levels or additional factors. Collectively, these data demonstrate

that MedRC - together with myeloid cells - not only form physical niches for PC, but also secrete factors capable of mediating PC survival and function.

DISCUSSION

In the current study, we characterized a new fibroblast subset that we termed MedRC as it is present as reticular cell network throughout the medullary cords and is phenotypically and functionally distinct from TRC and MRC. MedRC form niche-like structures in which PC and innate immune cells reside. In addition, they are a rich source of various factors that can promote PB/PC migration, localization, survival and function. We therefore propose that MedRC are an important component of PC niches inside LN and thereby positively regulate early humoral immunity.

The combination of two surface markers, MAdCAM and BP3, allowed us to distinguish murine MedRC from TRC and MRC. This classification was largely confirmed by intracellular staining for CCL21 and CXCL13 proteins that were absent in MedRC, but allowed a further functional subdivision of TRC and MRC at the single cell level. Around 60% of TRC express CCL21, consistent with previous observations (10, 20, 29, 30). TRC also comprise around 15% CXCL13^{+}CCL21^{-} cells, explaining the high level of *cxcl13* transcripts observed by us and others among pdpn^{+} FRC (20, 31), as well as *cxcl13* expression still found in follicles of FDC-depleted mice (32). Given the follicular localization of CXCL13 protein, these cells are likely to reside in the outer B cell follicle (21) and possibly co-express BAFF (22), the Notch-ligand DL-4 (33) and enzymes generating the chemoattractant oxysterol (20). We propose to call them 'B zone reticular cells' (BRC)(SI Appendix Fig. S7E). Our unbiased flow cytometric clustering analysis (TSNe) points to a total of at least 7 phenotypically distinct fibroblast-like cell types inside LN, not including the different CD35+ FDC types and Pdpn-CD31- (double negative or DN) fibroblastic cells that were gated out from this analysis. Notably, these 7 cell clusters appear to form a continuum that could reflect the zones as well as transitions between zones where specific processes, such as T-B collaboration or PB differentiation may occur (2, 34). A recent report based on single cell RNA sequencing of lymph node fibroblasts points to nine transcriptionally distinct populations, including the Inmt+ stromal cells which

most likely correspond to our MedRC subset (35). The exploration of these presumably different cell types or cell states, their relationship and regulation as well as their respective function will be an important area for the future.

The MedRC subset described here is number-wise a relatively large population within naïve and activated LN and has previously been considered together with their T zone counterpart. They include as a subset the recently described CD34+ perivascular FRC with precursor potential (30, 35). Importantly, MedRC express little T and B zone chemokines, while producing CXCL12 at levels comparable to TRC, consistent with high CXCL12-reporter expression in most LN FRC (24). Given the prevalence of CXCL12 expression in sorted MedRC compared to other MC cell types, along with the lacking expression in innate immune cells (8), we propose that MedRC are the major local source of this chemokine along with blood vessels (24). MedRC may therefore contribute to the medullary localization of $CXCR4^{+}$ cells which do not express much CCR7 and CXCR5, including PC (2, 14), granulocytes and macrophages, thereby generating the distinct cellular composition of MC. $CXCR4^{-/-}$ PB/PC fail to localize appropriately within MC (2), suggesting that cells excluded from T and B cell zones need CXCL12 cues to either migrate deeper into the MC or to be locally retained in this region. Consistent with it, pertussis toxin treatment of mice reduced PC accumulation in MC but most PB migration appeared to be non-directional, similar to previous evidence (28). *In vitro*, PB migrate towards CXCL12 gradients in a pertussis-toxin dependent manner (2, 14). Therefore, we propose that PB which have reached the outer T zone are either attracted or retained by the CXCL12-expressing MedRC. Additional factors, like accessible matrix fibers enriched within MC (36), may also contribute to PB/PC adhesion and retention.

Fooksman and colleagues have described the persistent linear tracks of PB in the LN T zone followed by the slower and much more confined migration of PB/PC once

they have reached MC (28, 37). We confirm these findings, and propose that the different migration strategy can be explained at least in part by PB/PC being physically guided initially by the TRC and later by the MedRC network, similar to the reported naïve lymphocyte migration along TRC and FDC (17). The molecular basis for this PB crawling along the FRC networks remains to be defined, but may include ICAM-1 on FRC (28). While the clearly decreased displacement of PC relative to PB is probably due to their terminal differentiation and therefore intrinsic changes, our results suggest that both the spatial organization and morphology of MedRC may also contribute. The FRC network is denser in MC than T zones. Relative to TRC, MedRC are less reticular and more stellate, presumably because they enwrap less the matrix fibers (36). This arrangement might impede fast and linear PC migration, with the more accessible matrix potentially allowing increased PC adhesion.

The slow PC motility within MC goes along with the long contact duration and large contact zones with MedRC. The cavities of the MedRC network appear tailor-made in size for allowing residence and signal recognition by single PC. Therefore, the term 'survival niche' seems very appropriate. This niche also includes B220^{+} B cells and myeloid cells, with the role of macrophages and dendritic cells being controversially discussed (4, 8, 10-12). We have observed synergistic effects on PC survival when combining MedRC with myeloid cells. This is in contrast to macrophage depletion experiments showing an enhanced extrafollicular PC response (12). We have also performed preliminary experiments to deplete MedRC in a CCL19Cre x ROSA-DTR cross (in collaboration with Lucas Onder and Burkhard Ludewig). This approach that depleted 50-75% of MedRC was associated with a 30-50% reduction in PC, and a strong reduction in IgM and IgG secretion by LN-resident PC. In addition, PC accumulated within the outer T zone suggesting MedRC and/or TRC are important for late B cell differentiation and/or PC survival. However, most LN cell types were affected by this depletion, including most TRC and thereby presumably also lymphocyte differentiation processes within T zones. Therefore, in

future, rather than using depletion systems, mouse models need to be developed to selectively ablate expression of PC guidance and survival factors in MedRC versus TRC. Despite the current lack of appropriate mouse models, the many lines of evidence presented here are all consistent with the notion that MedRC contribute significantly to PC niches inside LN, both structurally and functionally. We propose a model in which the PC niche is composed of motile hematopoietic cells but also of a static fibroblast network (SI Appendix Fig. S7F), reminiscent of bone marrow niches for long-lived PC (4, 5). This mixed composition may increase the robustness of the humoral response while allowing a more elaborate regulation. Whether fibroblasts also contribute to PC-niches in other tissues, including spleen and intestinal lamina propria, remains an interesting open question.

IL-6, BAFF, APRIL and CXCL12 can promote PC survival *in vitro* (3, 4), while less is known on their relative role and expression *in vivo* (24, 38). Analysis of various sorted innate cells has failed to identify the source of BAFF and CXCL12 (8) consistent with bone marrow chimera experiments pointing to non-hematopoietic cells as BAFF source (9). We now provide strong evidence for MedRC being the elusive source of both BAFF and CXCL12 within MC, with BAFF expression being highly MedRC restricted both at the mRNA and protein level, consistent with a recent report (34). This BAFF source is distinct from the less prominent one in the T and B zones. BAFF expressed by MedRC is very likely to be sensed by the neighboring PC enhancing their survival, while TRC-derived BAFF could contribute to the earlier differentiation and survival of B cell blasts. These two roles for BAFF are different than the recently proposed one for BRC-derived BAFF in naive B cell survival and follicular homeostasis (22). Our attempts at neutralizing BAFF during an ongoing response led to only a weak reduction in PC survival, comparable to the one recently reported for early PC responses in mice deficient in hematopoietic April or IL-6 (38).

Myeloid cells were proposed to be a strong source for IL-6 (8) but surprisingly their depletion increased PC numbers (12). We found higher IL-6 expression in MedRC than myeloid cells, both at the transcriptional and protein level, similar to a recent report (34), with MedRC being slightly more efficient in promoting IgM/G secretion by PC, again in an IL-6 dependent manner. While lymphoid tissue fibroblasts were known as potent IL-6 source that can mediate survival of PC and hybridoma cells (7, 14, 15) the fibroblast subset colocalizing with PC has to our knowledge not been sorted and tested ex vivo without passaging, as performed in this study. IL-6 is also known to regulate PC responses *in vivo*. However, previous publications have focused on late PC responses which most likely involve germinal center responses (6, 39, 40). Here we describe a 50% loss of the early extrafollicular PC generation in the absence of IL-6. Our reverse bone marrow chimera experiments using IL-6-/- and wt mice have failed to reveal any difference in the contribution of hematopoietic versus non-hematopoietic cells. These findings are consistent with our co-cultures, where both MedRC and myeloid cells promoted PC responses almost entirely via IL-6. While these findings establish that IL-6 produced by these cells can positively regulate PC survival and function, several signals were lost during culture, including BAFF and APRIL. It is also conceivable that the expression of negative regulators by myeloid cells was lost upon culture, which would explain the difference with some of the *in vivo* findings (12). Overall, our results are consistent with earlier proposals that *in vivo* PC survival may be controlled by a combination of factors, including IL-6, BAFF and APRIL (3, 4). The precise roles of BAFF, APRIL and IL-6 need to be further investigated *in vivo* using cell type-specific knockout mice, but the simultaneous presence of several B cell differentiation and PC survival factors as well as several niche cell types indicates a potential redundancy and robustness of the system (SI Appendix Fig. S7F).

While most of our study focused on mouse tissues, we have provided here first evidence for a similar fibroblast-based niche in MC of human LN. The markers described here and in a previous study (26) to distinguish human fibroblast subsets should be useful in future to sort and functionally define these cells, both in LN and sites of chronic inflammation where PC have been observed, like in rheumatoid arthritis joints or kidneys of lupus patients. Interestingly, in these settings fibroblast-like cells in the lesions have been established as major source of PC survival factors (41, 42), reminiscent of LN MedRC and TRC described in this study. In several B cell-mediated autoimmune diseases antibody-producing PC are thought to reside in large numbers inside the spleen and LN, presumably in stable niches and contributing to pathogenesis. In such disease types, treatment with anti-CD20 antibodies (rituximab) often does not efficiently deplete PC (43, 44). In contrast, therapies based on blocking BAFF/APRIL (Atacicept) or IL-6 have proven to be effective in reducing autoantibody levels and thereby autoimmune pathology (45, 46). These findings suggest that fibroblastic cells or their products are an interesting therapeutic target. Using this approach, pathological immune effector cells could be eliminated by destroying their survival niche. Conversely, boosting PC niches within LN, spleen or bone marrow may be a way of enhancing vaccine-induced humoral responses and memory.

EXPERIMENTAL PROCEDURES

Mice and Immunization

C57BL/6 mice were from Harlan Olac. OT-I and OT-II mice from the Jackson laboratory (bred to CD45.1$^+$ B6 mice). Other mice used were pCol-GFP transgenic (27), IL-6 knockout (39), BAFF knockout (47), non-cleavable BAFF transgenic (48), *plt/plt* (16). One million cells from LN and spleen of OT-I and OT-II mice were injected i.v. into recipient mice which were immunized the following day s.c. with 50

µl OVA/Mont (1 mg/ml ovalbumin (OVA, Sigma) containing 25% of Montanide ISA 25 (Seppic, France)) above areas drained by inguinal, axillary, and brachial LN (6 injections per mouse) (25). Alternatively, mice were immunized the same way with 50 µl of sheep red blood cells (SRBC) (Eurobio, France) leading to a comparable PC response. All mouse experiments were authorized by the Swiss Federal Veterinary Office (authorization number 1612.2) and by the animal experimentation ethic committee of University Paris Descartes, Paris, France (CEEA34.ED.042.12).

FRC isolation

Peripheral LN (pLN; axillary, brachial and inguinal) were dissected from sacrificed mice, cut into small pieces and digested for 30 min at 37°C with gentle stirring in 1.5 ml DMEM medium (Gibco) containing collagenase IV (3 mg/ml; Worthington), DNAse I (40 µg/ml; Roche), $CaCl_2$ (3 mM) and 2% (vol/vol) FCS (16). Cells were then passed through a 40 µm strainer (Becton Dickinson), washed twice and resuspended in DMEM/2% FCS.

Flow cytometry and cell sorting

Cells were labeled as described previously (25) using Abs listed in SI Appendix Table S1. Intracellular staining of chemokines was performed on surface marked cells fixed with 2% PFA, then permeabilized and stained in 0.1% saponin in PBS. Rabbit anti-CCL21 and Goat anti-CXCL13 were detected with Alexa 488 or 647 coupled secondary antibodies. Data were acquired on a LSRII (BD Biosystems) and analyzed with FlowJo (TreeStar). Cells were sorted with a FACSAria Ib for RNA isolation directly into lysis buffer (RNeasy micro kit, Qiagen) or for cell culture into complete RPMI medium (Gibco).

MACS purification and survival assays of plasmablasts and plasma cells

pLN of mice (OVA/Mont d6.5) passed through a 40 µm mesh were depleted of naïve

lymphocytes by panning (αB220, αTCRβ), then cell suspensions stained with anti-CD138 PE or biotin followed by anti-PE or Streptavidin magnetic beads, respectively, and finally enriched by magnetic-activated cell sorting (MACS; purity of 90-95%). Occasionally, they were labelled with 5nM CSFE before the enrichment. Sorted stromal or hematopoietic cells were cultured overnight in 96-well plates followed by adding purified PC at approximately a 5-10 fold higher number (1.2×10^4 or 6×10^4) and if indicated inhibitors: αIL-6 (MP5-20F3, BioXcell), αAPRIL (Apry-1-1, Adipogen), αAPRIL and αBAFF (hTACI-Fc, Adipogen) or AMD3100 (inhibitor of CXCR4, Sigma). After 3 days, surviving PC numbers were determined by FACS.

ELISA measurements of OVA-specific IgM and IgG, and of IL-6 protein levels

Flat-bottom 96 well MaxiSorp plates (Nunc) were coated with 5 µg/mL OVA and blocked with 1% BSA in PBS. Serial dilutions of samples were added for 1 h and bound antibody detected with biotinylated goat anti-mouse IgM or IgG (Invitrogen) followed by HRP-conjugated streptavidin (Jackson). Color development using TMB solution (Sigma) was measured at 450 nm with correction set at 550 nm. Relative antibody concentrations correspond to the dilution giving an OD of 50%. IL-6 protein level was measured in the supernatant of cultured cells, according to the kit's instructions (eBioscience).

RNA isolation and quantitative real-time PCR

RNA was extracted using the RNeasy micro kit (Qiagen). First-strand cDNA synthesis, quantitative real-time PCR, primers and normalization were as described previously (16) or shown in SI Appendix Table S2.

Immunofluorescence labeling of tissue sections

For murine tissue blocks, cryosections (8 µm) and labeling of naïve or OVA/Mont

activated pLN were as previously described (16). For details on antibodies see the SI Appenix Table S1. Human pLN were obtained from transplant patients (ethics permit: CA/5192) and 5 μm cryostat sections acetone-fixed before staining. See SI appendix for more details.

In situ **hybridization**

The Ccl19, Ccl21, Cxcl13, and Cxcl12 'riboprobe' was cloned and used as described (16, 49). The nucleotides 35 to 767 of *Il6* (NM_031168.1) were used as a template for making a riboprobe. Cryosections of pLN were treated as previously described (16). After *in situ* hybridization, tissue sections were stained with antibodies to B220 and laminin followed by fluorochrome-labeled secondary antibodies.

Microscopy and image analysis

Images were acquired either with an upright Zeiss Axioplan, a Zeiss SP5 confocal or a Leica DMI6000 spinning-disk confocal microscope. Images were treated using ImageJ (NIH), Adobe Photoshop or Imaris (Bitplane). Imaging of fibrillar collagen within LN of pCol-GFP mice was performed using SHG microscopy. To detect FRC-PC contacts, the colocalization tool in Imaris was used. Three-dimensional (3D) reconstruction of sequential *z* series was performed using the surface tool of Imaris. For additional information see SI appendix.

Imaging of plasma cell motility within live LN slices

Live vibratome sections were stained for 15 minutes at 37°C with APC-conjugated anti-CD138 (281.2) and washed thereafter. In some experiments, pCol-GFP mice were treated with either IL-6 neutralizing (MP5-20F3, 10μg/mL) or control antibodies at day 2 and day 5 after SRBC immunization. For details see SI appendix.

Statistical analysis

Statistical analysis was determined with an unpaired two-tailed Student's t-test or one way ANOVA by Prism 5 (GraphPad). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Detailed experimental procedures can be found in the SI appendix.

SUPPLEMENTAL INFORMATION APPENDIX

Supplemental information includes additional experimental procedures, seven figures, seven movies, two additional tables, references, and can be found as pdf file with this article online.

AUTHOR CONTRIBUTIONS

S.A.L., E.D. and H-Y.H. designed the study; H-Y.H., F.R., H.C., L.S., K.S., S.F., T.K.V. and S.A.L. performed research, with all live imaging experiments performed by A.R.-C., E.P, A.P. and E.D.; D.L.H and C.D.B. analyzed human lymph nodes; P.S. and F.A.-S. provided mice, reagents and/or advice; H-Y.H. and S.A.L. wrote the manuscript with important input from E.D..

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FIGURE LEGENDS

Fig. 1. Histological identification and characterization of a new FRC subset colocalizing with plasma cells in medullary cords of activated lymph nodes. Immunofluorescence microscopy of sections from pLN of naïve or OVA/Mont-immunized mice (**A-D**), or on sections from human pLN (**E-G**). (**A and B**) Cryostat sections stained for FRC (pdpn^{+}LYVE1^{-}), lymphatic vessels (pdpn^{+}LYVE1^{+}), CD3^{+} T cells, B220^{+} B cells and CD138^{+} PC. (**C-D**) Vibratome and cryostat sections of LN stained with indicated antibodies. (**E-G**) Sections of human pLN were stained for the indicated markers, with a focus on lymphoid compartments and vascular cells (**E**), fibroblastic cells (**F**) and extracellular matrix components (**G**). Boxed areas are shown at higher magnification in the right or the row below. Dashed lines indicating examples of MC where PC reside. Arrows point to representative FRC within a given zone. B, B cell zone (follicle); T, T cell zone; M, medulla; MC, medullary cords, MS, medullary sinus, SCS, subcapsular sinus; scale bar, 100µm. Results are representative for at least three independent mice or human samples.

Fig. 2. Medullary FRC have a unique phenotype and cytokine profile and expand in number during lymph node swelling. (**A-D**) FACS analysis of CD45^{-}CD35^{-} cells from pLN of naive or OVA/Mont-immunized mice at the indicated time points. (**A**) Dot plots in upper row show the gating of FRC (pdpn^{+}CD31^{-}) versus BEC (pdpn^{-}CD31^{+}) and LEC (pdpn^{+}CD31^{+}) from naïve pLN. The expression of MAdCAM and BP3 in FRC allows to distinguish three FRC subpopulations: MRC, TRC, and MedRC. Shown on the right side is the control staining when anti-MAdCAM antibody was not added. Dot plots in lower row show a representative staining of the 3 FRC subsets in naïve versus activated LN, with the percentages indicated for of each population (n≥3). (**B**) Number of total FRC and of each of the three FRC subsets per pLN upon immunization. (**C)** Representative histograms showing the level of BrdU incorporation in the three FRC subsets (OVA/Mont d5.5). Numbers indicate the percentage of

BrdU$^+$ cells. Black line: anti-BrdU antibody; dashed line: isotype control antibody on total FRC. (**D**) Representative histograms displaying the frequency of CCL21- and CXCL13-expressing cells among the three FRC subsets found in activated pLN. Black line: specific antibody; dashed line: no primary antibody on total FRC. (**E**) The three FRC subsets and CD31$^+$ cells (LEC/BEC) were sorted from activated pLN and normalized transcript levels of indicated cytokine transcripts measured by qRT-PCR (means±SD, *n*=4). (**F**) ISH analysis for indicated cytokine transcripts (green) or the sense control (for CXCL12). (**G**) pLN sections stained with the indicated antibodies. The boxed areas are represented below at higher magnification. (**H**) Multiparameter flow cytometry based clustering of FRC (CD45-31-pdpn+) subsets from naïve pLN using the TSNe algorithms (Flowjo) to display 7 parameters (FCS, SSC, Pdpn, BP3, MAdCAM-1, CXCL13 and CCL21) in a 2D representation. Colored areas were added manually. Scale bar, 100µm. Results in A-D and F-G are representative for at least three independent mice. *p<0.05, **p<0.01, *** p<0.001.

Fig. 3. Medullary FRC produce a dense matrix network and only partially associate with it. (**A**) Immunofluorescence microscopy of activated pLN (OVA/Mont d8.5) for the indicated antibodies. The boxed area is represented in the middle panel at higher magnification and with single colors. On the right side is shown a vibratome section of the LN medulla. (**B**) The levels of collagen 1α1 and collagen 1α2 transcripts are shown for the indicated cell types isolated from either naive or activated (OVA/Mont d5.5) pLN (means±SD, *n*=4). (**C-E**) Confocal images of vibratome sections of pLN (SRBC d7) from pCol-GFP mice and stained with the indicated antibodies. (**D**) Histogram showing the fluorescence intensity of each marker along the dashed line shown on middle panels. (**E**) The boxed areas are represented below at higher magnification and showing only the GFP$^+$ fibroblasts either in T zone or medulla. Graphs on the right show the GFP fluorescence intensity of FRC found in these two zones, their concentration, as well as the width and spacing between fibroblast

protrusions. (**F**) Two-photon microscopy images of naive and activated pLN (SRBC d7) from pCol-GFP mice showing GFP expression and SHG highlighting collagen fibers. (**G**) 3D reconstruction of two-photon images displaying T zones and MC of naive LN. See also SI Appendix Movie S1. (**H**) Vibratome section of pLN (OVA/Mont d5.5) 10 min after s.c. injection of Texas Red (TR)-dextran and stained with the antibodies indicated. The boxed areas are represented on the right at higher magnification.

Fig. 4: Medullary FRC are a major component of the plasma cell niche. (**A-B**) Immunofluorescence images of activated pLN with MC areas being highlighted. (**A**) Confocal microscopy of activated pLN (SRBC d7) from pCol-GFP mice stained with the antibodies indicated. Right: Frequency of PC in contact with F4/80^{+} and B220^{+} cells. Values are mean ± SD of 3-5 experiments in which conjugates were scored from 6-9 LN slices. (**B-C**) Localization and migration of endogenous PB/PC in vibratome sections of viable activated pLN from pCol-GFP mice. (**B**) Tracks of individual endogenous PB/PC in relation to GFP^{+} FRC. Tracks are color coded according to the extent of PB/PC displacement. Graphs on right side show velocities and displacement lengths of PB/PC. Values are from 3 experiments in which cells were monitored from 3-5 LN slices. See also SI Appendix Movie S3. (**C**) Motile behavior of PB/PC relative to GFP^{+} FRC. Top panels: Snapshots are shown at various time intervals for the same zone and cells. The trajectory of PB/PC in the outer T zone was superimposed over fluorescence images. See also SI Appendix Movie S4 and S5. Bottom left panel: Graphic representation of interactions between PB/PC and GFP^{+} FRC. Each row corresponds to an individual PB/PC, and the periods of contact with FRC are colored with different shades of green corresponding to different FRC partners and white corresponding to no FRC partner being visible. Graph on bottom right side summarizes the average contact time of PB/PC with their FRC partners.

Fig. 5. Medullary FRC express high levels of plasma cell survival factors. Mice were immunized with OVA/Mont and the expression of PC survival factors analyzed in draining pLN. (**A**) The transcript levels of *baff*, *april, il6 and cxcl12* was assessed in cells sorted from fully digested pLN as shown in Fig. 2A and SI Appendix Fig. S5A. T/B lymphocytes were $CD19^+$ or $TCR\beta^+$. (means±SEM, naïve *n*=2, OVA *n*=4). (**B**) Immunofluorescence staining of activated pLN from wt, uncleavable BAFF transgenic and BAFF KO mice for BAFF expression (red) on FRC and LEC in medulla. Arrows point to $BAFF^+$ TRC and MedRC. Stars indicate staining artifacts due to high endogenous HRP in BAFF KO mice. *n*=3. (**C**) ISH analysis of *il6* transcript or the sense control. The boxed areas are represented at higher magnification in the row below. Scale bar, 100µm. *n*=3. (**D**) ELISA for IL-6 protein levels in the supernatant of the indicated cell types sorted from d5 ova/Mont immunized pLN and cultured for 18h. Values were normalized for 10'000 cells. n=4-9. Number of experiments: 1 (A), 2 (D), 2-4 (B, C).

Fig. 6: IL-6 drives plasma cell survival but not homing in activated lymph nodes. Cell suspensions of pLN from wt or IL-6 KO mice (OVA/Mont d0, d5.5 or d8.5) were either analyzed ex vivo by FACS for the number of PC and intracellular IgG^+ PC per pLN (**A**) or for the titer of OVA-specific IgM/G measured in the supernatant of total pLN cells cultured for 3 days (**B**), n=7-13. Localization (**C**) and motility (**D**; velocity and displacement length) of endogenous PC/PB were monitored by time-lapse confocal microscopy in live vibratome slices from activated (SRBC d6) pLN of pCol-GFP mice treated either with neutralizing antibodies to IL-6 (anti-IL-6) or control antibodies (Ctrl). TZ, T zone; n=6 mice. Number of pooled experiments: 6 (A-B), 2 (C, D). See also SI Appendix Movie S7 and Fig. S6.

Fig. 7: Medullary FRC, together with macrophages and dendritic cells, promote PC survival with a major role for IL-6. Activated pLN (OVA/Mont d5-6) were used for MACS-isolation of PB/PC and FACS-sorting of potential support cells. PB/PC were cultured for 3 days in the indicated conditions followed by the analysis of surviving PCs by FACS ($B220^{low/-}CD138^{+}CD44^{+}IgM^{-}FSC^{hi}SSC^{hi}$) or of IgM/G antibody titers by ELISA. (**A**) Number of surviving PC after culture in presence of rIL-6, a monolayer of total FRC from d5.5 activated pLN (act.FRC), conditioned medium (CM) from total act.FRC cultured for 3 days, or in presence of a transwell separating act. FRC from PC. (**B**) Number of surviving PC after culture in presence or absence of GM-CSF (to improve myeloid cell survival) or of a layer of the indicated cells. (**C-E**) PC number and IgM/G titers after cocultures in presence or absence of IL-6 neutralizing antibody or rIL-6; with or without MedRC (C); macrophages (Mac) or DC (D); in presence or not of GM-CSF along with the indicated cell type(s) (E). Gr, granulocytes. Error bars present SD (*n*=3-8); $^{*/\#}$ p<0.05, $^{**/\#\#}$ p<0.01, $^{***/\#\#\#}$ p<0.001; in (F): *, compared with PC cultured with medium only (first column); $^{\#}$, compared with PC cultured with MedRC/Mac/DC/Gr (last column). Shown results are representative for 2 (A, C-E) or 5 (B) independent experiments.

REFERENCES

1. Mueller SN & Germain RN (2009) Stromal cell contributions to the homeostasis and functionality of the immune system. *Nat Rev Immunol* 9(9):618-629.

2. Cyster JG (2005) Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu Rev Immunol* 23:127-159.

3. Oracki SA, Walker JA, Hibbs ML, Corcoran LM, & Tarlinton DM (2010) Plasma cell development and survival. *Immunol Rev* 237(1):140-159.

4. Tangye SG (2011) Staying alive: regulation of plasma cell survival. *Trends in immunology* 32(12):595-602.

5. Zehentmeier S*, et al.* (2014) Static and dynamic components synergize to form a stable survival niche for bone marrow plasma cells. *Eur J Immunol* 44(8):2306-2317.

6. Cassese G*, et al.* (2003) Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. *J Immunol* 171(4):1684-1690.

7. Minges Wols HA, Underhill GH, Kansas GS, & Witte PL (2002) The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. *J Immunol* 169(8):4213-4221.

8. Mohr E*, et al.* (2009) Dendritic cells and monocyte/macrophages that create the IL-6/APRIL-rich lymph node microenvironments where plasmablasts mature. *J Immunol* 182(4):2113-2123.

9. Gorelik L*, et al.* (2003) Normal B cell homeostasis requires B cell activation factor production by radiation-resistant cells. *J Exp Med* 198(6):937-945.

10. Kumar V*, et al.* (2015) A dendritic-cell-stromal axis maintains immune responses in lymph nodes. *Immunity* 42(4):719-730.

11. Hebel K*, et al.* (2006) Plasma cell differentiation in T-independent type 2 immune responses is independent of CD11c(high) dendritic cells. *Eur J Immunol* 36(11):2912-2919.

12. Fooksman DR, Nussenzweig MC, & Dustin ML (2014) Myeloid cells limit production of antibody-secreting cells after immunization in the lymph node. *J Immunol* 192(3):1004-1012.

13. Minges Wols HA*, et al.* (2007) The effects of microenvironment and internal programming on plasma cell survival. *Int Immunol* 19(7):837-846.

14. Ellyard JI, Avery DT, Mackay CR, & Tangye SG (2005) Contribution of stromal cells to the migration, function and retention of plasma cells in human spleen: potential roles of CXCL12, IL-6 and CD54. *Eur J Immunol* 35(3):699-708.

15. Jourdan M*, et al.* (2014) IL-6 supports the generation of human long-lived plasma cells in combination with either APRIL or stromal cell-soluble factors. *Leukemia* 28(8):1647-1656.

16. Link A*, et al.* (2007) Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol* 8(11):1255-1265.

17. Bajenoff M*, et al.* (2006) Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* 25(6):989-1001.

18. Gretz JE, Anderson AO, & Shaw S (1997) Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol Rev* 156:11-24.

19. Katakai T, *et al.* (2008) Organizer-like reticular stromal cell layer common to adult secondary lymphoid organs. *J Immunol* 181(9):6189-6200.

20. Yi T, *et al.* (2012) Oxysterol gradient generation by lymphoid stromal cells guides activated B cell movement during humoral responses. *Immunity* 37(3):535-548.

21. Mionnet C, *et al.* (2013) Identification of a new stromal cell type involved in the regulation of inflamed B cell follicles. *PLoS biology* 11(10):e1001672.

22. Cremasco V, *et al.* (2014) B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. *Nat Immunol* 15(10):973-981.

23. Abe J, *et al.* (2012) B cells regulate antibody responses through the medullary remodeling of inflamed lymph nodes. *Int Immunol* 24(1):17-27.

24. Bannard O, *et al.* (2013) Germinal center centroblasts transition to a centrocyte phenotype according to a timed program and depend on the dark zone for effective selection. *Immunity* 39(5):912-924.

25. Yang CY, *et al.* (2014) Trapping of naive lymphocytes triggers rapid growth and remodeling of the fibroblast network in reactive murine lymph nodes. *Proc Natl Acad Sci U S A* 111(1):E109-118.

26. Link A, *et al.* (2011) Association of T-zone reticular networks and conduits with ectopic lymphoid tissues in mice and humans. *The American journal of pathology* 178(4):1662-1675.

27. Yata Y, *et al.* (2003) DNase I-hypersensitive sites enhance alpha1(I) collagen gene expression in hepatic stellate cells. *Hepatology* 37(2):267-276.

28. Fooksman DR*, et al.* (2010) Development and migration of plasma cells in the mouse lymph node. *Immunity* 33(1):118-127.

29. Luther SA, Tang HL, Hyman PL, Farr AG, & Cyster JG (2000) Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. *Proc Natl Acad Sci U S A* 97(23):12694-12699.

30. Sitnik KM*, et al.* (2016) Context-Dependent Development of Lymphoid Stroma from Adult CD34(+) Adventitial Progenitors. *Cell Rep* 14(10):2375-2388.

31. Malhotra D*, et al.* (2012) Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat Immunol* 13(5):499-510.

32. Wang X*, et al.* (2011) Follicular dendritic cells help establish follicle identity and promote B cell retention in germinal centers. *J Exp Med* 208(12):2497-2510.

33. Fasnacht N*, et al.* (2014) Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. *J Exp Med* 211(11):2265-2279.

34. Zhang YT, L.; George, L.A.; Acs, A.; Durrett, R.E.; Hess, H.; Walker, L.S.K.; Tarlinton, D.M.; Fletcher, A.L.; Hauser, A.E.; Toellner, K.-M. (2018) Plasma cell output from germinal centers is regulated by signals from Tfh and stromal cells. *J Exp Med* 215(4):1227-1243.

35. Rodda LBL, E.; Bennett, M.L.; Sokol, C.L.; Wang, X.; Luther, S.A.; Barres, B.A.; Luster, A.D.; Ye, C.J.; Cyster, J.G. (2018) Single-cell RNA sequencing

of lymph node stromal cells reveals niche-associated heterogeneity. *Immunity* 48:1014-1028.

36. Ushiki T, Ohtani O, & Abe K (1995) Scanning electron microscopic studies of reticular framework in the rat mesenteric lymph node. *The Anatomical record* 241(1):113-122.

37. Luther SA (2010) Plasma cell precursors: long-distance travelers looking for a home. *Immunity* 33(1):9-11.

38. McCarron MJ, Park PW, & Fooksman DR (2017) CD138 mediates selection of mature plasma cells by regulating their survival. *Blood* 129(20):2749-2759.

39. Kopf M, Herren S, Wiles MV, Pepys MB, & Kosco-Vilbois MH (1998) Interleukin 6 influences germinal center development and antibody production via a contribution of C3 complement component. *J Exp Med* 188(10):1895-1906.

40. Wu Y*, et al.* (2009) IL-6 produced by immune complex-activated follicular dendritic cells promotes germinal center reactions, IgG responses and somatic hypermutation. *Int Immunol* 21(6):745-756.

41. Noss EH & Brenner MB (2008) The role and therapeutic implications of fibroblast-like synoviocytes in inflammation and cartilage erosion in rheumatoid arthritis. *Immunol Rev* 223:252-270.

42. Wang W*, et al.* (2014) Long-term B cell depletion in murine lupus eliminates autoantibody-secreting cells and is associated with alterations in the kidney plasma cell niche. *J Immunol* 192(7):3011-3020.

43. Shlomchik MJ (2008) Sites and stages of autoreactive B cell activation and regulation. *Immunity* 28(1):18-28.

44. Mahevas M, Michel M, Weill JC, & Reynaud CA (2013) Long-lived plasma cells in autoimmunity: lessons from B-cell depleting therapy. *Frontiers in immunology* 4:494.

45. Mackay F & Schneider P (2009) Cracking the BAFF code. *Nat Rev Immunol* 9(7):491-502.

46. Kopf M, Bachmann MF, & Marsland BJ (2010) Averting inflammation by targeting the cytokine environment. *Nature reviews. Drug discovery* 9(9):703-718.

47. Schiemann B*, et al.* (2001) An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 293(5537):2111-2114.

48. Bossen C*, et al.* (2011) Mutation of the BAFF furin cleavage site impairs B-cell homeostasis and antibody responses. *Eur J Immunol* 41(3):787-797.

49. Hargreaves DC*, et al.* (2001) A coordinated change in chemokine responsiveness guides plasma cell movements. *J Exp Med* 194(1):45-56.