Perspective

Responses of Long-Lived Subsets and Diverse Plasma Cells to Microbiota, Autoantigen, and Immunization

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ABSTRACT

Long-lived plasma cells (LLPCs), which are largely found in the bone marrow, are necessary for the development of durable antibody protection (BM). However, due to the rarity of LLPC, neither their phenotypes nor their heterogeneity have been able to be identified. We demonstrate that IgG and IgM LLPCs exhibit an EpCAMhiCXCR3- phenotype, whereas IgA LLPCs are Ly6AhiTigit-, using single-cell mRNA sequencing, cytometry, and a genetic pulse-chase mice model. IgA and IgM LLPC compartments contain cells with innate characteristics and public antibodies in contrast to IgG and IgA LLPCs, which are mostly contributed by somatically hyper mutated cells after immunisation or infection. In particular, IgM LLPCs differentiate in a T cell-independent way, are substantially enriched with public clones shared among many individual animals, and have affinity for both self-antigens and microbial-derived antigens. Together, our research demonstrates various paths that LLPCs can take and opens the door to a greater comprehension of the cellular and molecular bases of long-lasting antibody protection.

Keywords: Plasma cells, Antibody protection, mRNA sequencing, Immunisation

Introduction

Plasma cells (PCs) are B cells that have undergone terminal differentiation and have been programmed to produce antibodies. Some of these cells adopt niches in the bone marrow (BM) after first differentiating in secondary lymphoid organs or inflammatory tissues and go on to live as long-lived PCs (LLPCs), lasting as long as the hosts do without repeated antigen stimulation. LLPCs serve as the foundation for vaccine-induced long-term protection and are significant elements of humoral immunological memory. Understanding LLPC distinction and maintenance is crucial. The scarcity of LLPCs makes research on them difficult. Less than 10 LLPCs produced by a specific vaccine can be identified in 1 ml of bone marrow (BM), and in people, LLPCs are only thought to make up about 25% of all bone marrow PCs (BMPCs). After lymphocytic choriomeningitis virus infection, the number of virus-specific LLPCs in the BM of mice is thought to be only 20,000–30,000. The fact that PCs in secondary lymphoid organs and BM seem to be a varied population made up of cells produced from numerous B cell progenitors through various activation routes and at various stages of differentiation adds to the problems. All of these PCs in mice express a common set of intracellular and surface markers, such as CD138, XBP1, BLIMP1 (encoded by prdm1), and IRF4. As a result, it is still difficult to distinguish genuine LLPCs from all other PCs, which is necessary to gain a deeper understanding of the cell biology that underlies their differentiation and maintenance.

Long-lasting LLPCs (LLPCs) often have B cell receptors (BCRs) that are highly altered and have greater affinities, indicating that they originated from germinal centres (GCs). It has been widely accepted that T cell assistance and affinity maturation during the GC reaction are essential for LLPC formation and survival. In the BM compartment, where comparable enrichment of LLPCs is anticipated, transcriptomic

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investigations of bulk splenic PCs and BMPCs at the population level have shown overexpression of genes involved in metabolism, chemotaxis, and cell-cell interactions. It is yet unclear whether and how GC experience might encourage those improvements, and it is also unclear whether of those changes specifically apply to LLPCs. The observations suggesting GC-independent LLPC production might potentially occur compound this ambiguity even more. B1 cells and traditional B2 cells are the two main lineages of mature B cells. B1 cells are innate-like B cells that form in the peritoneal and pleural cavities as well as solid organs during the foetal and neonatal stages of development. When compared to B2 cells, B1 cells have a different antigen receptor repertoire, which includes BCRs that preferentially rearrange themselves throughout early development and identify common self-antigens and microbial products. PCs can develop from both B1 and B2 lineages of B cells, although LLPCs are believed to be mostly produced from B2 cells, supporting the idea of GC dependency. Murine BM has PCs with B1-lineage signatures, although it is unclear if these PCs are LLPCs.

We have combined single-cell RNA sequencing (scRNA-seq) to analyse the transcriptomes and BCR repertoires of splenic PCs (SPPCs) and BMPCs in naive and immunised mice, immune profiling to identify subset-defining surface markers, and a genetic pulse-chase approach to estimate the half-lives of PC subsets in order to better characterise LLPCs and probe their heterogeneity and function. Our findings show that Ly6AhiTigit- and EpCAMhiCXCR3- surface phenotypes can be used to specifically identify mouse antigen-specific IgA and IgG LLPCs. We also present a subgroup of unmutated IgM LLPCs that, despite transcriptional similarities to IgG LLPCs generated by T cell-dependent antigens, develops in mice at rest in a T cell-independent manner. These cells are concentrated in public antibody sequences, such as those found in B1a cells that detect self-antigen and commensal microorganisms, and they are somewhat dependent on the microbiota.

Results

■ Differential SPPC and BMPC phenotypes

IgG LLPCs were largely produced as a result of this vaccination. The detection of PCs was aided by the bacterial artificial chromosome transgenic Blimp1-enhanced yellow fluorescent protein reporter strain. BCRs with a VH1-72 heavy chain and a -light chain were reactive to NP hapten1 in B6 mice, allowing us to sequence-track antigen-specific PCs. We examined two separate time series, sampling SPPCs and BMPCs from six mice in each condition, to determine the temporal sequence of PC production. The surface expression of CD138 on spleen (SP) and BM cells was used to enrich PCs, which were subsequently sorted and purified as CD138+eYFP+ cells. To process all samples, 10x Genomics performed scRNA-seq runs and four different library preparations. Across all time points and tissue types, 44,189 PCs with constructed BCRs were recovered in total. Automatic clustering divided PCs into 15 distinct clusters based on their transcriptomes after excluding transcripts for IgG after accounting for batch effects. By examining the uniform distribution of genes and unique molecular identifiers (UMIs) found per cell, we evaluated the quality of our datasets.

Identification of immunization-induced LLPCs

We concentrated our investigation on VH1-72++ NP-specific PCs produced in response to NP-KLH vaccination in order to find LLPCs among different PC clusters. In data from immunised mice, a total of 713 such cells were discovered, whereas 4 were discovered in data from naive mice. On the 15-cluster map, the bulk of NP-specific cells were primarily IgG switched and localised in C8 and C9. NP-specific cells showed a spatiotemporal trend of accumulation in C8 and depletion from C9 with time. The BM showed a clear tendency toward C8 accumulation, whereas the SP showed a clear trend toward C9 depletion. We counted the W33L affinity-enhancing mutation and discovered that by day 60, NP-specific BMPCs had the highest affinity for C8, but all SPPCs had the highest affinity for C9 cells. This finding suggests that throughout time, NP-specific cells in splenic C9 may give rise to C8 in the BM. This idea is supported by the fact that the three clusters C3, C7, and C8 had the highest concentrations of immunoglobulin transcripts. Second, genetic data suggests that the antiapoptotic proteins MCL1 and BCL2, the co-stimulatory receptor CD28, and the BCMA receptor are necessary for the long-term survival of LLPCs. All of these genes were found to be substantially expressed in C3, C7, and C8 cells, but Bcl2 was hardly expressed in the shorter-lived C11 IgA and C9 IgG PC subsets.

■ Surface phenotyping of LLPCs

We carefully examined the expression of genes encoding surface markers that may distinguish the 15 clusters in order to be able to recognise LLPC subsets in the BM or SP by surface phenotyping. Epcam and Cxcr3 were substantially expressed by the long-lived (C7, C8 and C12) and short-lived (C2 and C9) IgG and IgM cells, respectively, in the BM and SP. The relative higher expression of Ly6a and lower expression of Tigit in the long-lived C3 subset of IgA PCs allows for differentiation from the short-lived C11 group. By analysing SPPCs and BMPCs from naive mice using fluorescence-activated cell sorting (FACS), we discovered that most IgG and IgM PCs in both the BM and SP displayed EpCAM at a variety of levels on the cell surface.

Discussion

Our understanding of long-term antibody-mediated immune protection depends critically on PC lifespan, which appears to be controlled by both extrinsic and internal mechanisms. The long-lived potential of LLPCs is lost when they are removed from their microenvironment, which contains numerous immune and non-immune cell types that produce survival factors like interleukin-6 (IL-6), CXCL12, APRIL, BAFF, and CD80/CD86. The majority of immunization-induced LLPCs, on the other hand, are hypermutated and require the cytokine IL-21 produced by follicular helper T cells, suggesting that GC PC development may have partially imprinted lifespan. A transcriptomic programme underlying the longevity of LLPCs has been difficult to define, despite the fact that extrinsic factors crucial for LLPC survival are fairly well understood4. This is in part because the BM contains a variety of PCs with various inferred lifespans, but no surface markers have yet been able to separate these subsets. We have described PC subsets in the mouse SP and BM, evaluated their half-lives, and identified the long-lived populations by integrating single-cell sequencing, FACS analysis, and a genetic pulse-chase mouse model.

Our findings support the notion that PC longevity is guided by intrinsic transcription programmes in addition to being influenced by the microenvironment and metabolic changes experienced, and that PC longevity is associated with specific transcriptomic states that take shape relatively early during PC development.

BMPCs are movable but also form long-lasting connections with stromal cells, according to intravital imaging. IgG and IgM LLPCs are notable in this context for having high levels of EpCAM expression and low levels of the chemokine receptor CXCR3 expression. EpCAM has a role in the epithelial-to-mesenchymal transition, cancer metastasis, and homophilic adhesion between epithelial cells. EpCAM has also been demonstrated to support memory T cell survival when produced ectopically40. EpCAM may enable IgG and IgM PCs stay anchored to their distinct survival niches and relay signals that control their longevity, while CXCR3 may divert prospective LLPCs away from those niches. It's intriguing that IgA LLPCs are transcriptionally different from IgG and IgM LLPCs, possibly indicating that they have a different history of mucosal development. A recent transcriptome investigation of PCs reported the existence of isotype-specific characteristics in PC transcriptomes. Importantly, IgA LLPCs only express a moderate amount of EpCAM, but their Ly6AhiTigit characteristic makes them distinct from other IgA PCs. Both GC-dependent and GC-independent pathways could be used to reach the cell state shared by these two LLPC populations. Most interestingly, C7 IgM LLPC formation does not require T cell assistance, indicating that whatever survival benefit that may be provided by GC exposure may also be imprinted via a fundamentally different mechanism. C7's exclusivity in the public BCR repertoire shows that the precursor state and antigen being identified are crucial.

It is remarkable that different mice have identical clones of the C7 EpCAMhiCXCR3 IgM LLPC in their separate compartments, some of which include antibody sequences specific for phosphatidylcholine that had previously been found in B1a cells that are innately similar. Additionally, while EpCAMhiCXCR3 IgM BMPCs can recover after PC deletion when the microbiota is present as it should be, most of them cannot do so when the microbiota is eliminated by antibiotics, indicating that a sizable portion of these cells are capable of recognising microbiota-associated antigens. At least two C7 public clones that are convincingly able to distinguish between dead cells and intestinal bacteria support this theory. We also found clones in germline configuration with affinity for the microbiota in the IgA LLPC compartment, which lends further credence to this theory. All things considered, our findings show that

LLPC development from B cells with innate-like characteristics is possible via a T cell-independent pathway. We discovered that mouse BM contains a lot of IgM PCs, which is in line with earlier research. Contrarily, although IgM BMPCs are also present, human BMPCs are predominately IgA and IgG isotypes. This variation between the two species can be a result of their divergent evolutionary paths. As an alternative, it might originate from SPF mice's comparatively limited antigen exposure history. B1 cells might also be able to change into IgG and IgA isotypes and add to the IgA PC pool.

Future research is necessary to comprehend how innate-like B cells develop into LLPCs and how IgM BMPCs differ between species. As a result, a better mechanistic understanding may hold the key to enhancing our capacity to programme PCs with the desired specificities, isotypes, and longevity for the development of vaccines and the treatment of antibody-mediated diseases.