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New insights into heterogeneity of peritoneal B-1a cells

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Abstract

Peritoneal B-1a cells are characterized by their expression of CD5 and enrichment for germ line–encoded IgM B cell receptors (BCRs). Early studies showing expression of a diverse array of VDJ sequences among purified B-1a cells provided a molecular basis for understanding the heterogeneity of the B-1a cell repertoire. Antigen-driven positive selection and the identification of B-1a specific progenitors suggest multiple origins of B-1a cells. The introduction of new markers such as PD-L2, CD25, CD73, and PC1 (plasma cell alloantigen 1, also known as ectonucleotide phosphodiesterase/pyrophosphatase 1 (ENPP1)) further helped to identify phenotypically and functionally distinct B-1a subsets. Among many B-1a subsets defined by these new markers, PC1 is unique in that it subdivides B-1a cells into PC1^{hi} and PC1^{lo} subpopulations with distinct functions, such as production of natural IgM and gut IgA, response to the pneumococcal antigen PPS-3, secretion of interleukin (IL)-10, and support for T helper 1 (T_H1) cell differentiation. RNA sequencing (RNA-seq) of these subsets revealed differential expression of genes involved in cellular movement and immune cell trafficking. We will discuss these new insights underlying the heterogeneous nature of the B-1a cell repertoire.

Keywords

B-1a; PC1; IL-10; lymphocyte trafficking

Introduction

The mouse peritoneal cavity hosts three mature B cell subpopulations, namely B-1a (CD5⁺), B-1b (CD5⁻), and B-2 cells. The CD11b marker originally used to define B-1 cells (regardless of CD5 expression) was later found to only identify about 50% of peritoneal B-1

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Conflicts of interest

The authors have no conflict of interest.

cells (B-1a and B-1b),¹ which sheds doubt on the validity of using CD11b to mark B-1 cells in general. While B-1b and B-2 cells require constant input from developing bone marrow (BM) B cell precursors,^{2,3} B-1a cells are maintained by self-renewal throughout life. Differing from B-2 cells, B-1a cells have several distinctive characteristics: (1) they have higher basal BCR signaling activity than B-2 cells;^{4–6} (2) they respond to microbial products to differentiate into antibody-secreting cells more efficiently than B-2 cells;⁷ (3) they produce IL-10 and other cytokines and exhibit regulatory functions under steady-state conditions, while B-2 cells do not^{8,9} (B-2 cells may acquire regulatory functions after appropriate stimulation^{10–12}); (4) they have phagocytic activity reminiscent of macrophages, whereas B-2 cells do not;^{13,14} and, finally, (5) they are the major source (> 80%) of serum natural IgM antibodies (reviewed in Ref. 15). For years, these distinctive features have been assigned to all B-1a cells. However, the understanding of how these functions are manifested at the clonal level is rather limited.

Earlier studies using hybridomas generated from neonatal mice¹⁶ and PCR-based Ig gene sequencing of B cells from young mice¹⁷ (4 days to 4 weeks old) revealed little diversity of non-templated (N) regions in Ly1⁺ (CD5⁺) B-1a cells of newborn mice, owing to the fact that N sequence insertions are mediated by terminal deoxynucleotidyl transferase (TdT), which is not expressed in the liver during fetal life.¹⁸ However, N sequences are very common in peritoneal B-1a cells of 4-week-old mice,¹⁷ and about two-thirds of B-1a cells contain N sequences.¹⁹ These observations, in conjunction with unique V_H gene usage by individual B-1a cells,^{19,20} provide a structural basis for a highly diverse B-1a cell compartment. Indeed, distinct antigen specificities have been associated with specific B-1a subsets. For example, B-1a cells expressing V_H11/V_κ9 and V_H12/V_κ4 specifically bind to phosphatidylcholine (PtC);^{21,22} V_HS107-expressing B-1a cells react with phosphorylcholine (PC);^{23,24} and V_H3609/V_κ21C B-1a cells exhibit specificity for Thy-1.²⁵ More recently, the identification of B-1a cell progenitors, transitional B-1a cells, and different waves of B-1a cell generation through early life have shed new light on the developmental of a highly diverse B-1a cell repertoire.^{2,26–30}

Efforts to understand the heterogeneity of the B-1a compartment by several groups, including ours, have included studies of the expression of a number of cell surface antigens, including CD11b, CD25, D6, CD73, PD-L2, and PC1, on peritoneal B-1a cells. In this review, we will focus on the functional diversity of peritoneal B-1a cells defined by these newly identified markers.

B-1a cell subsets

Mouse peritoneal B-1a cells are defined as CD19^{hi}CD23[–]CD43⁺IgM^{hi}IgD^{lo/–}B220^{lo/–}CD5⁺.^{31,32} A combination of several of these markers is required to distinguish B-1a from B-1b and B-2 cells.³³ Over the past several years a variety of cell surface markers have been studied for their expression on B-1a cells, including CD25 (IL-2R α), D6, CD73, PD-L2, and PC1.

Rothstein and colleagues have reported CD25,³⁴ CD73,³⁵ and PD-L2³⁶ as potential markers that distinguish subpopulations of B-1a cells. These markers have been shown to expressed

on B-1a cells at frequencies of about 20–50%, depending on the marker.^{34–36} The extent of overlap among these markers is not yet known. These studies showed that CD25 is expressed by about 10% of peritoneal B-1b B cells in BALB/c mice, about half the frequency found for B-1a cells, and at levels about twice as high as on B-1a cells, according to the ImmGen database. Among CD25-defined B-1a subsets, high expression of CD25 is associated with elevated levels of phosphorylated STAT3 (pSTAT3), a downstream signaling molecule of cytokine receptors that was shown to be constitutively activated in B-1a, but not B-2, cells.³⁴ Interestingly, despite expression of CD25, IL-2 is presumably not responsible for the increase in pSTAT3, as B-1a cells do not respond to IL-2.³⁴ Instead, pSTAT3 is likely triggered through the leukemia inhibitory factor receptor (LIFR) on CD25⁺ B-1a cells.³⁴ Functional analyses of B-1a subsets defined by PD-L2 and CD73 revealed little difference between positive and negative subsets in IgM secretion, cytokine production (especially IL-10), activity in inducing T helper cell 17 (T_H17 differentiation), and responses to BCR and Toll-like receptor (TLR) stimulation.^{35,36} In autoimmune prone BXSB.Yaa mice, circulating PD-L2⁺ B-1a cell numbers are increased and may play a role in autoimmunity.³⁷

More recently, our group reported that PC1, also known as ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), can be used to subdivide B-1a populations.³⁸ PC1 is a transmembrane molecule with a documented enzymatic activity of cleaving ATP to generate AMP and inorganic pyrophosphate (PPi).^{39–41} PPi is a well-defined negative regulator of calcification/mineralization of bones.³⁹ Expression of PC1 is relatively ubiquitous, with high levels occurring in brain capillaries, chondrocytes, hepatocytes, epididymis, and salivary glands.⁴² Using a monoclonal anti-PC1 antibody and flow cytometry analyses, we found high expression of PC1 in neutrophils, germinal center B cells, and plasma cells.⁴³

Unlike the other markers discussed above, which revealed subtle if any functional differences among the subpopulations they define, PC1 expression distinguishes two phenotypically and functionally distinct populations, which we designated B-1a.PC1^{hi} and B-1a.PC1^{lo}. A largely non-overlapping Ig gene repertoire is used by both subsets, confirming their phenotypic distinction.³⁸ All the strains of laboratory mice tested to date have PC1^{hi} and PC1^{lo} B-1a subsets, with a ratio of about 1:2.³⁸ We therefore propose that PC1 might serve as a unique marker to further characterize the heterogeneous nature of the B-1a cell repertoire.

Multiple waves of development and antigen-driven clonal expansion contribute to B-1a cell diversity

Dorshkind and colleagues have recently proposed a model of B-1 cell development involving three waves of generation.²⁹ The first wave starts in the yolk sac and intra-embryonic para-aortic splanchnopleura tissues as early as embryonic day E9.⁴⁴ The second wave occurs at a later time in fetal liver and BM, where the B-1 progenitors differentiate into mature B-1a cells. The third wave of B-1a cell development occurs after birth in the BM, where hematopoietic stem cells (HSCs) predominantly give rise to conventional B-2 cells but continue to generate B-1a cells at a significantly reduced level. Earlier work in studying CD5⁺ B-1a cells with specificity for bromelain-treated mouse red blood cells⁴⁵ and

later studies in induced B-1a cell differentiation (reviewed in Ref. 46) are consistent with an antigen-selected third wave of B-1a cell differentiation.

By studying PC1-defined B-1a subsets in fetal and young mice, we found that, in addition to this progressive multi-wave developmental process, B-1a cells also exhibited clonal expansion at a young age. In E18 fetal liver, only 6% of CD5⁺ B-1a cells had a PC1^{hi} phenotype, and this frequency gradually increased with age, reaching plateau in 8-week-old adults with 34% of B-1a cells being PC1^{hi}.³⁸ This increase in PC1^{hi} cells in young mice could result from increased input from the third wave of BM production and/or *in situ* clonal expansion of PC1^{hi} cells of fetal origin. The former possibility is unlikely for the following reasons. First, the B-1 progenitors in the BM decline significantly in the weeks after birth.³⁰ Second, based on analysis of N region additions in *Igh* chains of 4-week-old mice when N sequences begin to become abundant,¹⁷ PC1^{hi} B-1a cells originating from BM progenitors of 3- to 5-month-old mice would be expected to contain high frequencies of N sequences. However, contrary to expectations, the overall frequency of N region additions at the V_H-D and D-J_H joins was significantly lower in PC1^{hi} than in PC1^{lo} B-1a cells.³⁸ The V_H11 and V_H5 sequences that dominate the PC1^{hi} subset mostly lack N sequence insertions.³⁸ While formal proof of clonal expansion requires more investigation, we speculate that environmental antigens from the normal biome acquired from the mother may drive this selection process. The segregation of PC1^{lo} and PC1^{hi} B-1a cells during different developmental stages is summarized in Figure 1.

Different homing capacities

Given that B-1a cells play a variety of innate functions, such as production of natural IgM in the spleen and IgA in the gut, a constant emigration of B-1a cells from the peritoneum is expected. The anatomical site of B-1a migration from and into the peritoneal cavity has been suggested to be omental lymphoid follicles (reviewed in Ref. 47). The omentum is a bilayered sheet of mesothelial cells connecting a large part of the intestine, stomach, pancreas, and spleen (reviewed in Ref. 48). Lymphocyte-rich follicles, also known as “milky spots,” express high levels of CXCL13, which attracts the migration of circulating CXCR5⁺ B-1a cells into the peritoneal cavity.⁴⁹ Expression of CCR7 and downregulation of adhesion molecules CD9 and integrins (e.g., α4, α6, and β1) are associated with egress of peritoneal B-1a cells.^{50,51} In addition, sphingosine-1-phosphate (S1P) is also involved in B-1a cell trafficking.⁵²

Peritoneal B-1a cells are maintained by self-renewal,^{3,53} thus differing from B-2 cells, which require continuous input from adult BM B cell progenitors. How self-replenishment is achieved remains poorly understood. Earlier studies of BrdU incorporation by total peritoneal B cells and with CD5⁺ B-1a cells demonstrated a slow turnover rate of ~ 1% per day.^{54,55} These studies showed no differences between B-1a and B-2 cells. This BrdU-labeling approach, however, is limited by cell cycle status and cannot determine where the cells became labeled. Recently, Labadi and Balogh reported an interesting *in situ* carboxyfluorescein succinimidyl ester (CFSE)-labeling method.⁵⁶ They injected mice intraperitoneally with 50 μg of CFSE dissolved in 1 ml of PBS. At various time points thereafter, the peritoneal cells were lavaged and CFSE-labeled cells were analyzed by flow

cytometry. This method permits the identification of newly immigrated B cells that are CFSE⁻ and cell divisions that are visualized as gradual reductions in CFSE intensities. Their data clearly showed a significant difference in turnover ratios between B-1 (B220^{lo}CD5^{+/+}CD11b⁺) and B-2 (B220^{hi}CD5⁻CD11b⁻) cells, with B-2 cells turning over strikingly faster than B-1 cells. The 50% turnover ratio was about 2 days for B-2 cells and 3 weeks for B-1 cells. In our study, we not only confirmed this result, but also revealed new features of the dynamic movement of peritoneal B cells.³⁸ We demonstrated that B-1b cells have an exchange rate intermediate between B-2 and B-1a. More intriguingly, the exchange rate for PC1^{hi} B-1a cells was significantly slower than for PC1^{lo} B-1a cells.³⁸ We also observed cell divisions of differing extents in different B cell subsets at a given time point.³⁸ In general, the rank of frequencies of dividing cells is B-2 > B-1b > B-1a.PC1^{lo} > B-1a.PC1^{hi}. Because PC1-defined B-1a subsets showed distinct exchange kinetics in the peritoneum, we further investigated their homing capacities through adoptive transfer via intraperitoneal or intravenous routes. Seven days after intravenous transfer, both subsets homed to the peritoneum and some to the spleen at equivalent levels. Strikingly, 2 months after intraperitoneal transfer, B-1a.PC1^{hi} cells were nearly completely restricted to the peritoneum, whereas B-1a.PC1^{lo} cells were localized not only to the peritoneum but also to the spleen and mesenteric lymph nodes.³⁸ These data strongly suggest that the B-1a.PC1^{hi} cells are less mobile than the B-1a.PC1^{lo} cells under steady-state conditions. Our work in progress has also shown that both subsets rapidly emigrated from the peritoneum upon treatment with lipid A (Wang and Morse, unpublished observation), similar to what was reported for lipid A-stimulated B-1a cell relocation.^{50,57}

Different capacities to produce innate-like antibodies

While B-1a subsets defined by CD25, CD73, D6, and PD-L2 showed no differences in production of IgM antibodies,^{34–36,58} PC1-defined B-1a subsets differed in their production of natural antibodies.³⁸ When adoptively transferred into the peritoneum of *Rag1*^{-/-} mice, the B-1a.PC1^{lo} cells produced significantly more circulating IgM than the B-1a.PC1^{hi} cells.³⁸ This was associated with their different tendencies to home to the spleen,³⁸ a site known for B-1a differentiation into plasma cells.^{57,59} Gut IgA originated exclusively from the B-1a.PC1^{lo} subset.³⁸ The inability of the B-1a.PC1^{hi} cells to become IgA secreting is quite striking because the donor B-1a.PC1^{hi} cells survived in the peritoneum for at least 6 months, indistinguishable from the B-1a.PC1^{lo} donors. The reasons for this phenomenon are currently unknown.

In addition to differing in their production of natural IgM and IgA antibodies, B-1a B cell subsets also showed distinctly different capacities to produce antigen-specific antibodies. In this case, *Rag1*^{-/-} mice were reconstituted with either subset of B-1a cells and then immunized intraperitoneally with type-3 pneumococcal polysaccharide (PPS3), a protective immunogen of *Streptococcus pneumoniae*. The PPS3-specific IgM antibodies in serum were induced exclusively in the B-1a.PC1^{lo} recipients.³⁸ These data suggest that the B-1a.PC1^{lo} cells are enriched for the PPS3 specificity and are selectively activated by PPS3 in a T cell-independent manner. We currently hypothesize that this anti-PPS3 response is BCR dependent because of similar findings in studies of a small fraction of B-1a cells that are able to respond to *Francisella tularensis* LPS in a BCR-dependent fashion.^{60–62}

Different capacities to activate T cells

It has been broadly accepted that B-1a cells have immunoregulatory functions that directly affect T cells. B-1a cells have been shown to provide help to T cells through antigen presentation,⁶³ accessory molecules CD80 and CD86,⁶⁴ and cytokines such as IL-6, IFN- γ , IL-4, and TGF- β .^{65–66} Differing combinations of these factors results in differentiation of T cells into effector cells with features of T_{reg}, T_{H1}, or T_{H17} lineages, depending on the stimulation condition.^{38,65–67} More importantly, peritoneal B-1a cells constitutively express IL-10 mRNA⁸ (using an IL-10–GFP reporter) under steady-state conditions.³⁸ The production of IL-10 is further increased following stimulation with TLR ligands such as LPS.^{8,66} Tedder and colleagues estimated that about 30% of peritoneal B-1a cells are IL-10 producers.⁶⁸ Adoptive transfer of peritoneal B-1 cells into recipient mice has been shown to suppress development of contact hypersensitivity⁶⁹ and colitis.^{35,70,71} In these models, IL-10 appears to be a critical mediator because transfer of IL-10–deficient B-1a cells largely abrogates their suppressive activity.^{35,69,70} Therefore, we speculate that, under physiological conditions, continuous secretion of IL-10 by B-1a cells may curb inflammation caused by gut microflora, thereby contributing to stomach and intestinal homeostasis.

Interestingly, we could segregate IL-10–producing B-1a subsets based on levels of PC1 expression. Following stimulation with LPS, IL-10–producing cells were greatly enriched in the B-1a.PC1^{hi} subset, compared with B-2, B-1b, and B-1a.PC1^{lo} cells.³⁸ We confirmed this observation by using IL-10–GFP reporter mice and found that the GFP⁺ cells were exclusively B-1a.PC1^{hi} cells.³⁸ These data are consistent with the hypothesis that B-1a.PC1^{hi} cells, which are mostly permanent residents of the peritoneum, are more important in stomach and intestinal homeostasis than the B-1a.PC1^{lo} subset.

Among the recently defined B-1a subsets, little or no difference was found in their abilities to induce T cell polarization.⁶⁷ However, by co-culturing OT-II–primed PC1-defined B-1a subsets with OT-II TCR transgenic T cells, we demonstrated a clear difference between B-1a subsets, in that B-1a.PC1^{hi} cells induced significantly more IFN- γ –producing cells than did the B-1a.PC1^{lo} cells. Interestingly, both subsets induced equivalent levels of T cell proliferation.³⁸ These data again indicate that PC1^{hi} and PC1^{lo} cells behave like separate populations.

Molecular signatures of B-1a subsets

To better understand the molecular basis of B-1a cell diversity, we have analyzed gene expression profiles of the B-1a.PC1^{hi} and B-1a.PC1^{lo} subsets using next-generation RNA sequencing (RNA-seq). We identified 81 genes that were differentially expressed between the two subsets with a false discovery rate–adjusted *P* value of $< 10^{-5}$. As shown in Tables 1 and 2, 58 genes were downregulated and 23 genes were upregulated in B-1a.PC1^{hi} cells compared with B-1a.PC1^{lo} cells. Strikingly, a large proportion of these genes (~40%) have functions related to cellular movement and immune cell trafficking (Table 1 and 2, genes indicated with an asterisk). Ten downregulated genes in the B-1a.PC1^{hi} subset, including *CXCR3*, *CCR7*, *CCL22*, *ANXA1*, *LDLR*, *LTTAF*, *LRRK2*, *SERPINB1*, *SOCS3*, and *TREML2*, are directly involved in chemotaxis. We validated the *CXCR3* expression differences by

staining with an anti-CXCR3 antibody followed by flow cytometric analysis (Wang and Morse, unpublished data). The finding that CXCR3 expression is higher on PC1^{lo} cells that migrate and become secretory is in keeping with the finding that CXCR3 expression and responsiveness to CXCR3 ligands is increased for migratory human and mouse plasmablasts during secondary immune responses.^{72,73}

Ingenuity pathway analyses of the differentiated genes further revealed upstream regulators, including LPS, TNF, IL-10, and IL-1 β . These observations suggest that, under steady-state conditions, these soluble factors constantly trigger downstream signaling cascades responsible for maintaining B-1a cell phenotypes and localization. These data are consistent with previous reports that bacterial products are strong modulators for peritoneal B-1a cell movement and function,⁵⁰ and that IL-10 modulates the sensitivity of B-1a cells to other chemokines.⁷⁴ It is worth noting that the gut microbiome appears to constantly promote B-1a cell trafficking, as germ-free mice have almost twice as many B-1a cells in the peritoneum as specific pathogen-free controls⁵⁰ (and Wang and Morse, unpublished data).

Concluding remarks

B-1a cells are an important element of the innate immune system. Since their discovery in 1983,⁷⁵ the mechanisms underlying their multiple functions, e.g., in antimicrobial infection, autoimmunity, and homeostasis, have remained, at best, incompletely understood. There is now accumulating evidence that B-1a cells as a whole are generated from different developmental waves that encompass the long period from fetal to young adult life. In adults, under certain circumstances such as stress, B-1a progenitors that are generally dormant in the spleen can be activated to replenish the B-1a cell pool.²⁷ All these events contribute to the “layered” B-1a cell repertoire.

Our findings, based on defining PC1^{hi} and PC1^{lo} B-1a subsets, not only confirms the notion of “layered immunity” but also extend our understanding of a functionally diverse B-1a cell compartment. The innate functions of B-1a cells, such as production of natural IgM and gut IgA, secretion of IL-10, and responses to bacterial antigens, can be differentially assigned to PC1^{hi} and PC1^{lo} subpopulations (Fig. 2). RNA-seq analysis has further revealed the molecular basis underlying this difference: that the two subsets are distinguished by their different gene expression programs, especially by expression of genes controlling cellular movement and trafficking. Future studies should focus on the underlying mechanisms for this phenotype.

Materials and methods

RNA sequencing (RNA-seq)

Peritoneal washout cells of 20 mice were pooled, stained, and sorted for B-1a.PC1^{lo} and B-1a.PC1^{hi} cells using a BD FACS-Aria machine and the gating strategy depicted in Figure 1. RNA processing, library construction, and deep sequencing using an Illumina HiSeq 2000 platform were described in detail previously.⁷⁶ Sequenced reads were aligned to the RefSeq mouse gene database (mm8 revision). Genes that were differentially expressed were identified with the statistical R package edgeR with a false discovery rate-adjusted *P* value

$< 10^{-5}$. The selected genes were then analyzed using Ingenuity IPA software. The data were derived from two independent experiments.

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Figure 1.

PC1^{lo} and PC1^{hi} B-1a cell development. B-1 progenitors (B-1p) of fetal liver mostly differentiate into B-1a cells (> 90%) with a phenotype of PC1^{lo}. These cells persist throughout neonatal and adult stages by self-renewal. However, BM B-1p of neonates and young adults continue to generate B-1a.PC1^{lo} cells, albeit at a lower efficiency than fetal B-1p (Wang and Morse, unpublished data). The generation of B-1a.PC1^{hi} cells begins in fetal liver but increases in neonates and reaches a plateau in adults. The low frequencies of N nucleotides observed in the PC1^{hi} B-1a repertoire argues for minimal contribution of BM B-1p in neonates and adults. Thick line, strong bias of lineage commitment/selection.

Figure 2.

Segregation of B-1a cell functions. (A) Most documented B-1a cell functions that are listed have been ambiguously regarded as intrinsic characteristics of all or most B-1a cells. (B) PC1 expression in PC1^{lo} and PC1^{hi} B-1a subsets segregates B-1a cell functions. This functional heterogeneity is supported by differential expression of gene programs involved in signal transduction and cellular movement.

Table 1Downregulated genes in B-1a.PC1^{hi} cells

Gene symbol	Log ₂ (FC)
<i>Csrp2</i>	-6.56
<i>Cp</i>	-6.47
<i>Ltk</i>	-6.20*
<i>Emp1</i>	-6.17
<i>Amhr2</i>	-6.16
<i>Mtss1l</i>	-6.06
<i>Csrnp3</i>	-5.85
<i>Aqp3</i>	-5.80*
<i>Pla2g4a</i>	-4.20*
<i>Afap1</i>	-3.65*
<i>Slc1a4</i>	-3.59
<i>Phxr4</i>	-3.14
<i>1300014I06Rik</i>	-3.06
<i>Zap70</i>	-2.99*
<i>Cxcr3</i>	-2.92*
<i>Pvr1l</i>	-2.74
<i>Folr4</i>	-2.42
<i>Anxa1</i>	-2.16*
<i>Havcr1</i>	-2.14
<i>Cpm</i>	-2.12
<i>Trpm5</i>	-2.07
<i>Satb1</i>	-1.96*
<i>Dlgap5</i>	-1.88
<i>AI504432</i>	-1.78
<i>Lrrk2</i>	-1.77*
<i>Trem12</i>	-1.70*
<i>Neurl3</i>	-1.68
<i>Gpr174</i>	-1.60
<i>Pdzd2</i>	-1.58
<i>Ccl22</i>	-1.54*
<i>BC017612</i>	-1.51
<i>Kif13a</i>	-1.49
<i>Vopp1</i>	-1.36
<i>Gpr171</i>	-1.36
<i>Ldlr</i>	-1.34*
<i>Cpd</i>	-1.33
<i>Fbxw10</i>	-1.32

Gene symbol	Log ₂ (FC)
<i>Socs3</i>	−1.25*
<i>Hdac9</i>	−1.24*
<i>Ms4a4c</i>	−1.19
<i>Sh2d2a</i>	−1.16*
<i>Cd72</i>	−1.16*
<i>Chd3</i>	−1.07
<i>Serpib1a</i>	−1.04*
<i>Amigo2</i>	−1.00
<i>Matk</i>	−0.99*
<i>Lck</i>	−0.97*
<i>Scn4a</i>	−0.96
<i>Vcl</i>	−0.92*
<i>Syt11</i>	−0.91
<i>Pgap1</i>	−0.89
<i>Adam19</i>	−0.83*
<i>Inpp5f</i>	−0.81
<i>Ccr7</i>	−0.80*
<i>Med13</i>	−0.80
<i>Dennd4a</i>	−0.68
<i>Ephx1</i>	−0.66
<i>Gpr183</i>	−0.62*

* Functionally involved in cellular movement and immune cell trafficking. FC, fold change.

Table 2Upregulated genes in B-1a.PC1^{hi} cells

Gene symbol	Log ₂ (FC)
<i>Litaf</i>	0.61 *
<i>Ass1</i>	0.62
<i>Gm5424</i>	0.62
<i>Anxa5</i>	0.65 *
<i>Rap1gap2</i>	0.69
<i>Als2</i>	0.71 *
<i>Pdcd1lg2</i>	0.74 *
<i>Csf2rb2</i>	0.75 *
<i>Slc39a4</i>	0.77
<i>Adm</i>	0.79 *
<i>Grb7</i>	0.96 *
<i>Ccr9</i>	0.99 *
<i>Zcwpw1</i>	1.14
<i>Fam174b</i>	1.17
<i>Pilra</i>	1.22 *
<i>Syn3</i>	1.38
<i>Scamp1</i>	1.55
<i>Rgl3</i>	1.71
<i>Kndc1</i>	1.86
<i>Cbr2</i>	3.88
<i>Celsr2</i>	5.64
<i>Soat2</i>	6.09
<i>Gng4</i>	6.23

* Functionally involved in cellular movement and immune cell trafficking. FC, fold change.