Isotype-specific plasma cells express divergent transcriptional programs

Brett W. Higgins*, Andrew G. Shuparski†, Karen B. Miller§, Amanda M. Robinson*, Louise J. McHeyzer-Williams*□, and Michael G. McHeyzer-Williams*†

Antibodies are produced across multiple isotypes with distinct properties that coordinate initial antigen clearance and confer long-term antigen-specific immune protection. Here, we interrogate the molecular programs of isotype-specific murine plasma cells (PC) following helper T cell-dependent immunization and within established steady-state immunity. We developed a single-cell–indexed and targeted molecular strategy to dissect conserved and divergent components of the rapid effector phase of antigen-specific IgM\(^+\) versus inflammation-modulating programs dictated by type 1 IgG2a/b\(^+\) PC differentiation. During antibody affinity maturation, the germinal center (GC) cycle imparts separable programs for post-GC type 2 inhibitory IgG1\(^+\) and type 1 inflammatory IgG2a/b\(^+\) PC to direct long-term cellular function. In the steady state, two subsets of IgM\(^+\) and separate IgG2b\(^+\) PC programs clearly segregate from splenic type 3 IgA\(^+\) PC programs that emphasize mucosal barrier protection. These diverse isotype-specific molecular pathways of PC differentiation control complementary modules of antigen clearance and immune protection that could be selectively targeted for immunotherapeutic applications and vaccine design.

plasma cell | isotype | antibodies | single-cell RNA-seq

In response to T-dependent (TD) antigen, antigen-presenting B cells are activated by follicular helper T cells (T\(_{FHI}\)) (1, 2), and undergo class-switch recombination (CSR) through cytokine-driven signaling cascade events (3–5). As CD4\(^+\) T helper cells and innate lymphoid cells are categorized into subsets based on discrete immune function (6, 7), B cells can be similarly assorted by antibody class (8, 9). IgM\(^+\) antibody is secreted as a pentamer for avidity-based antigen neutralization, complement activation, or labeling for phagocyte uptake. Each secreted antibody elicits separable immune responses through class-specific F\(_r\) receptor binding, such as type 1 inflammation induction by IgG2a/b\(^+\) subclasses and type 2 inflammation reduction by IgG1\(^+\) antibody (10–12). IgA\(^+\) antibody can be uniquely produced as a dimer wrapped by a secretory component for translocation across epithelial layers to provide type 3 mucosal barrier defense and enable commensal microbiota tolerance (13–15). The role of plasma cells (PCs) extends beyond antibody secretion as IgM\(^+\) and IgA\(^+\) PCs have been linked with interleukin (IL)-10, IL-17, IL-35, and tumor necrosis factor (TNF)-\(\alpha\) secretory activity (16–20). Thus, PC immune effector activity is divided by antibody isotype; however, there remains little resolution of the divergent transcriptional control of immune module function across these class-specific PC compartments.

PC terminal differentiation requires expression of a conserved transcriptional program, guided by the up-regulation of master transcriptional regulators Blimp-1, Xbp1, and IRF4 with concurrent down-regulation of B cell lineage-driving factors, such as Bcl6, Pax5, Pu.1, and IRF8 (21–29). During antigen clearance, an initial wave of PC differentiation produces an effector cohort that survives only days, secreting low-affinity antibody for antigen control and Fc receptor-driven immune regulation. This early immune response module is influenced by T\(_{FHI}\) cell driven CSR, which produces varied PC antibody classes. Subsequently, B cells enter a germinal center (GC) reaction under the cognate direction of T\(_{FHI}\) cells to undergo affinity maturation and exit as post-GC “memory” PCs capable of long-term survival for durable antigen-specific protection (30–34). During this memory phase of the immune response, T\(_{FHI}\) cell-directed GC cycling likely imprints supplementary molecular programming necessary for post-GC PC function and survival. It remains unclear the extent to which CSR and GC cycling impact the expression of ancillary molecular programs that divide these PC cohorts functionally at the single-cell level.

Targeted immunization with model antigen and the unimmunized “steady-state” murine models provide access to varied classes of PC. Infectious models can be used together with antigen-binding to isolate antigen-specific PC for molecular analysis of

Significance

During infection, antibodies are produced to target the rapid clearance of initial pathogen with high-affinity variants used long term to protect against reinfection. Under the guidance of CD4\(^+\) T cell regulation, different subtypes of antibodies emerge expressing separable molecular clearance mechanisms. Here, we study the transcriptional programs imprinted upon vaccine-induced single isotype-specific plasma cells specialized for early short-lived antibody production, long-term production of high-affinity antibodies, and splenic plasma cells persistent at steady state. We reveal divergent transcriptional programs across these phases of adaptive immunity and between antibody isotype that differentially influence pathways for antibody production, intercellular communication, cellular homing, and longevity. Understanding the cellular distribution of these molecular differences will help target and enhance isotype-specific immune effector outcomes through vaccination.
transcription (35). Priming with self-antigen can drive production of antiself-antibodies of different isotypes that can have a protective function against autoimmune tissue damage (36). TD antigen NP-KLH (4-hydroxy-3-nitrophenylacetyl conjugated to keyhole limpet hemocyanin) synchronizes intact immune systems in vivo, allowing access to pre-GC and follicular B cells for single-cell molecular analysis (37, 38). Using NP-KLH and Toll-like receptor (TLR)4 agonist MPL as an adjuvant, we have detailed an antigen-specific IgG2a/b+ and IgG1+–dominated response with the effector pre-GC phase consisting of an unmutated PC compartment at day 5, and a post-GC phase by day 14, where all PCs exhibit somatic mutation (8, 38–40). In contrast, an unimmunized steady-state murine system continuously exposed to environmental and gut microbiota antigen presents an ongoing, polyclonal immune response composed of IgM+, IgA+, and IgG+ B cell classes. As a result, the steady-state system provides the capacity to compare isotype-specific PC subsets distributed across multiple lymphoid organs (13, 41, 42). Combining the use of these two models enables the interrogation of discrete molecular programming expressed by each class and subclass of PC.

Here we use a quantitative gene-targeted single-cell RNA-sequencing (RNA-seq) strategy (43, 44) to interrogate the underlying transcriptional heterogeneity of class-specific PCs. We demonstrate that antigen-specific effector IgM+ and inflammatory IgG+ PC subclasses segregate transcriptionally with divergent molecular programs acquired during the GC reaction. Using a TLR4 inflammatory mediator, we reveal waves of type 1 antigen-binding antibody isotype that react to initial adjuvant exposure, convert towards type 2 antiinflammatory IgG1 with divergent transcriptional programs, following GC maturation and post-GC PC differentiation. This divergence was recapitulated in the steady state by IgM+, IgG2b+, and IgA+ PCs, which exhibited unique and defining molecular programs that extended further into two phenotypically and transcriptionally distinct subpopulations within the IgM+ compartment. These studies indicate that divergent transcriptional programs are imprinted according to broad immune response modules in both effector and post-GC pathways of PC differentiation. We propose that differential isotype-specific PC programming can be selectively targeted for immune therapeutic modification and future vaccine design.

Results

Antigen-Driven Segregation of Isotype-Specific PC Differentiation. Acute response PC compartments express antigen-binding B cell receptor (BCR) following helper T cell-dependent immunization at both the extrafollicular effector PC phase (day 5) and the post-GC PC differentiation phase (9, 23, 40) (day 14) (Fig. 1 A and B and SI Appendix, Fig. S1 A–F). These flow cytometry-based isolation strategies capture the majority of local PC activity in vivo, as seen by antigen-binding and isotype-specific ELISPOT (Fig. 1 C and SI Appendix, Fig. S1 G–I). Expression of both CD138 and intracellular Blimp-1 protein attest further to the identity of antigen-specific IgM+ and class-switched PC at the effector and post-GC memory PC phase in the draining lymph nodes (dLN) and bone marrow (BM) (Fig. 1 D and SI Appendix, Figs. S1 D and E). Cell cycle indicator Ki67 varied across antigen-specific PC compartments with highest levels at day 5 in both IgM+ and class-switched compartments (Fig. 1 D). Bimodal distributions of Ki67 at day 14 post-GC represents time since GC exit and terminal differentiation both locally and within the BM. As expected, expression of the B cell isofrom of CD45, B220, and the BCR coreceptor CD19 decreased differentially by location and time but additionally by class, highlighting the potential for programmatic divergence in vivo (Fig. 1 D).

IgM+ PCs represented only a minor fraction of responders to this TD antigen and MPL adjuvant. In contrast, among the IgG subclasses, type 1 inflammatory IgG2a+ and IgG2b+ emerged rapidly to dominate by day 5, accompanied by IgG1+ at day 7 with equivalent numbers of this type 2 antiinflammatory isotype present post-GC by day 14 (Fig. 1 E and F and SI Appendix, Fig. S1 F). This antigen-specific PC differentiation model provides experimental access to early effector PCs expressing IgM+ and temporally and developmentally separate IgG+ subclasses to interrogate for transcriptional divergence at the single-cell level.

Divergent IgM and IgG Effector PC Programs. As immunoglobulin mRNA makes up over 70% of sequenced cDNA species, using global sequencing approaches in PCs (29, 45, 46) we developed a more targeted single-cell RNA-seq protocol for these studies (43, 44). This quantitative and targeted RNA-seq strategy (single-cell qT-seq) begins with high-dimensional FACS sorted and indexed antigen-specific PC that are tracked and processed as single cells (Fig. 2 A). We currently target ~500 gene products designed to exclude immunoglobulin genes but focus on expressed mRNA for transcriptional modifiers translocating to or residing in the nucleus (n = 152), cell surface molecules (n = 203), and cytoplasmic and secreted species (n = 91 and n = 51, respectively). Across this target set, barcoding was designed to identify reads from each individual sorted cell with the capacity to quantify per cell expression using unique molecular identifiers (UMIs) in a similar manner to global approaches (SI Appendix, Fig. S2 A). Dimensionality reduction of cell-surface phenotype and gene expression distinguished the index-sorted effector IgM+ and IgG+ PC compartments (Fig. 2 B and C). Relying on statistically significant divergence in gene expression, expression fold-change (Fig. 2 D–F), and averaged heatmap representations of scaled signals in heatmaps (Fig. 2 G), we establish multiple isotype-segregating components of the IgM+ and class-switched antigen-specific effector PC transcriptional program.

The loss of central naive B cell transcriptional programs (Bcl6, Pax5, Bach2,Spi) were a cardinal feature of early effector IgM+ PC differentiation (Fig. 2 D and SI Appendix, Fig. S2 B). These substantial changes in transcriptional regulation were accompanied by increased PC lineage drivers (e.g., Prdm1, Irf4, Xbp1, Pou2af1), with some factors expressed significantly higher in extrafollicular IgM+ effector PC compared to their class-switched counterpart (Fig. 2 D and E and SI Appendix, Fig. S2 C). Multiple other transcriptional drivers were differentially expressed at higher (Skil, Ncor2, Pou2af2, Plac8, Nfat2) or lower levels (Smad4, Traf3, Pparg, Bhlha15, and Bhlha41) to allow separation of the central programs of IgM+ from IgG+ class-switched PCs at the effector stage (Fig. 2 F and G). IgM+ PCs may express ancillary effector functions related to differentially expressed secretory molecules (e.g., Tgfβ2, Ltb3, Il1a), in contrast to IgG+ PC from this stage (e.g., Il15, Sibs, S100a9) (Fig. 2 F and G). Finally, there are significant differences in expression of surface effector molecules, including chemokine receptors and cell-adhesion mediators between PC subsets (e.g., Ccr1, Tiafr513b, Slamf7, Cila4, and Cat17αa in IgM+; Timd2, Il9r, Cxcr3, and Sele in IgG) (Fig. 2 G). This programmatic heterogeneity can markedly impact class-specific BCR responsiveness, immunomodulation, migratory behavior, and survival
requirements. Together, IgM+ and IgG+ effector PC compartments demonstrate divergent transcriptional programs with evidence for separable secretory and surface modifiers that direct class-specific immune function.

**Inflammatory Subclass IgG Effector PC Programs.** Closer scrutiny of IgG2a+ and IgG2b+ effector PC (Fig. 3A and SI Appendix, Fig. S3) revealed further significant transcriptional differences between the cells expressing these early inflammatory mediating antibody subclasses (Fig. 3 B–D). Expression of major transcriptional regulators are significantly higher in IgG2a+ PC (FosJ1, Klf6, Klf7, Bcl6b, and Ifj1) in contrast to IgG2b+ PCs (Ptparg, Bhlha15, Bhlhe41, Xbp1, Ifnjf1) (Fig. 3 D and E). Differential up-regulation of secretory molecules that have been linked with eliciting inflammatory responses (Il22, S100a9, and Gzma) in IgG2a+; Il15 in IgG2b+ as well as surface-expressed proteins (Tnfrsf1b, Tnfrsf9, Tnfrsf14, and Gzmb in IgG2a+; Haver2 in IgG2b+) further distinguished a separable IgG2a+ transcriptional module from IgG2b+ PCs. Consistent with the inflammatory functions of IgG2a/b, subclass Cxcr3 is expressed highly compared to IgM and validated with protein expression. Surface expression of MHC-II by effector PCs suggested conserved retention of antigen-presentation capacity, though differential Cxcr3 and B220 expression emphasized shared programmatic components of IgG+ subclasses that still differed from IgM+ PCs (Fig. 3 F–H). The separation of antigen-specific effector PCs into IgG2a+ and IgG2b+ subclasses emphasizes the existence of dichotomous subclass-linked molecular programming with evidence for heterogenous inflammatory response control.

**Divergent Isotype-Specific Post-GC PC Programs.** The GC cycle is fully operative by day 14 of this immune response, producing antigen-specific memory B cells and post-GC PCs that express affinity-matured BCR (8, 37–40, 47–50). This established GC reaction produces equivalent numbers of antiflammatory IgG1+ post-GC PC and their inflammatory IgG2a+ and IgG2b+ post-GC PC counterparts (Figs. 1E and 4 A and B). PCs from day 14 were all sorted at the same time, including IgG1+, IgG2b+, and IgG1–IgG2b– cells. Compared to naive B cells, post-GC IgG1+ PCs up-regulated master transcriptional regulators involved in cell cycling, antibody secretion and UPR control (e.g., Ube2c, Mki67, E2f2, E2f2, Xbp1, Asf1a, Bhlha15), cytokine-linked secretory components (e.g., Ifng, Il15, Il22), and surface molecules (e.g., Cd44, Igal, Cd93, Cd28, Cd80, Cd276, Cd9) that direct long-term cellular function (Fig. 4 C–E).
Fig. 2. Divergent IgM and IgG effector PC programs. (A) Flow cytometry of index-sorted antigen-specific IgM+ (Upper, in green) and IgG+ (Lower, in blue) PCs overlaid on contour plots of total populations. (B) Dimensional reduction on index sorted PCs based on surface phenotype using tSNE (see Materials and Methods for details) or (C) based on gene-expression distribution using UMAP (see Materials and Methods for details). (D and E) From the index-sorting data, the known cell identity was overlaid in color (naive B cells grey, day 5 antigen-specific IgM+ PCs green and IgG+ PCs blue) after the tSNE or UMAP plot generated. (D) Differences in gene expression plotted by statistical significance and presented as volcano plots comparing naive B cells (CD19+ IgM+ PCs, in green) and IgG+ PCs, or between (F) antigen-specific IgM+ and IgG+ PCs. Significant gene-expression differences for naive B cells (colored grey), day 5 IgM+ PCs (green), and IgG+ PCs (blue). All expression with a fold-change less than 0.5 log₂ and P > 0.05 (negative binomial) are colored black. (G) Pseudobulk heatmap representation of differentially expressed genes separated by designated gene location of function (nucleus represents transcriptional modifier translocation to or residing in the nucleus genes, surface membrane, and cytosol and secreted genes) from D–F using average gene expression values in each cell population. The color scale (scale bar: blue [low] through yellow to red [high]) is based on z-score. For D–G, index-sorted cells n = 73 naive B cells, 109 antigen-specific IgM+, and 269 antigen-specific IgG+ cells.
Comparison between post-GC PCs indicated IgG1+ post-GC PCs expressed significantly higher levels of central transcriptional regulators (Spib, Bach2, Pou2af1, Creb3l2, Irf4, El2) that would coordinate separable programs upon GC exit. In contrast, IgG2a+ post-GC PCs differentially expressed a separate set of regulators (Irf1, Kmt2d, Relb, Nfatc2) that segregate further against IgG2b+ post-GC PC (Hif1a, Klf4, Klf6, Bhlha15, Plac8, Batf, Notch1, Prdm1, and Aif4) (Fig. 4 F–I). Surface-expressed, cytosolic, and secreted components of these programs begin to indicate the impact of the transcriptional differences on subclass-specific post-GC PC function. IgG1+ post-GC PC differentially expressed multiple molecules with capacity to elicit divergent function (Plexnd1, Tr3, Cd24a, Blk, Lamp1) (Fig. 4 F and I), while IgG2a+ post-GC PCs exhibited unique gene up-regulation, including a set of secretory molecules (Isg15, Ltbp3, Ilia) associated with directing a range of intercellular functions (Fig. 4 G and H). In the context of the targeted gene set, IgG2b+ post-GC B cells differentially expressed multiple segregated programs, notably a series of cellular adhesion molecules (Cd9, Cd81, Cd80, Ppnc, Tiget, Slamf9) (Fig. 4 H and I). These divergent transcriptional changes remain prominent and persistent even following the complex behavior of somatic hypermutation and antigen-driven selection that is paramount in the GC reaction.

As described above, we used K67 as a marker of recent cell cycling and hence as a proxy for recent GC cycle exit in post-GC PCs. We utilized index tracing to divide post-GC PCs by Mki67 expression and determined that each IgG subclass contained equal frequencies of Mki67+ cells (SI Appendix, Fig. S4B). As expected of cells more recently cycling, Mki67+ PCs were more transcriptionally active with enrichment of programs associated with apoptosis, cell division, and replication compared to the Mki67− population (SI Appendix, Fig. S4 C and D).
Fig. 4. Divergent isotype-specific post-GC PC programs. (A) Flow cytometry contour plots of total populations from day 14 postimmunization with index-sorted antigen-specific IgG1* (grey), IgG2a* (red), and IgG2b* (blue) PCs overlaid. (B) tSNE generated plot using the FlowJo tSNE tool of all index-sorted antigen-specific day 14 PCs from the LN. The clustering used protein surface-expression levels (excluding lineage-negative fluorophores) with FlowJo preset conditions and automatic learning configuration. Based on the index-sorting data, the known cell identity was overlaid in color (IgG1* grey, IgG2a* red, and IgG2b* blue) after the tSNE plot generated \((n = 290 \text{ IgG1}\, +, 228 \text{ IgG2a}\, +, \text{ and } 292 \text{ IgG2b}\, + \text{ PCs})\). (C–E) Differences in gene expression plotted by statistical significance and presented as volcano plots comparing naive B cells (CD19* IgD* IgM* λ1* CD138*/CD0* Gr1*/CD0* CD3e*/CD0, colored dark grey) and antigen-specific IgG1* PCs (colored light grey) separated by gene location of function (nucleus, cytosol and secreted, and surface membrane). (F–H) Differences in gene expression plotted by statistical significance and presented as volcano plots comparing (f) IgG2b or IgG2a antigen-specific PCs (colored purple) to antigen-specific IgG1* (light grey), (g) IgG1 or IgG2b antigen-specific PCs (colored purple) to antigen specific IgG2a* (red), and (h) IgG1 or IgG2a antigen-specific PCs (colored purple) to antigen specific IgG2b* (blue). Expression with a fold-change less than 0.5 log₂ and \(P > 0.05\) (negative binomial) are colored black. (I) Pseudobulk heatmap representation of differentially expressed genes from F–H using average gene-expression values. The color scale is based on \(z\)-score distribution. Genes are arranged by location of function (nucleus, surface membrane, or cytosol and secreted) \((n = 78 \text{ naive B cells, } 290 \text{ IgG1}\, +, 292 \text{ IgG2b}\, +, \text{ and } 228 \text{ IgG2a}\, + \text{ antigen-specific PCs})\).
Separable Inflammatory Post-GC PC Programs. While post-GC PCs retained expression of MHC-II similar to effector PCs, they exhibit differential expression of CXCRI3 and FAS (Fig. 5A). Furthermore, gene program-driven dimensionality reduction separates effector and post-GC PCs (Fig. 5B). Through comparison of inflammatory class effector PCs to post-GC PCs, we can establish unique molecular programs imprinted by GC cycling (Fig. 5 C and D). Dividing our analysis into IgG2α and IgG2β subclasses resolved subclass-specific transcriptional programs that emerge post-GC (Fig. 5 E–G). The reliance on highly influential modules of transcriptional regulation at day 5 (Myx, Foxj1, Pena, and Notch2 in IgG2a; E2f2, Gita, Bhlha15, Pparg, Akt1, Smad4, Ifi4, and Ulbe2c in IgG2b) was significantly shifted by day 14 in the post-GC PC compartment, which up-regulated a separate series of nuclear factors (Relb and Knt2d in IgG2a; Klf7 and Ski1 in both; Stat6 and Mef2c in IgG2b) (Fig. 5G).

Beyond transcriptional regulation there were significant changes in expression of important cell membrane-expressed regulators of immune function that extended further to segregation by antibody subclass; we revealed changes from day 5 effector PCs (e.g., Tnfrsf1b, Tnfrsf18, and Il1r2, in IgG2a; Cxcr3, Cd24a, Ly6d, and Cd24a in IgG2b) to post-GC PCs (e.g., Cd40l, Cx22, and Tnfrsf11 in IgG2a; Il1rn, Cd80, Slamf9, Cd86, and Cd93 in IgG2b). Post-GC PCs also up-regulated secretory factors (Lisp3, Il1a, Sipi, Cx3b, Ifng, Il21r) not represented in effector PC compartments. These subclass-specific components of post-GC changes—including chemokine receptors, B7 family receptors, and adhesion molecules—suggest shifts in cellular function imprinted by the GC program prior to post-GC PC differentiation.

Divergent IgM and IgG2b PC Programs in the Steady State. Next, we chose to broaden our molecular analyses to the range of isotype-specific PC found in the steady-state spleen. While CD138 is a reliable PC marker in the context of antigen-expressing B cells, we isolated IgM− IgM+ class-switched B cells served to identify antibody-secreting PC of separable isotype (Fig. 6A and SI Appendix, Fig. S5 A and B). The IgM+ and IgG2b+ subsets broadly separated in Uniform Manifold Approximation and Projection (UMAP) clusters based on the full range of gene expression from qtSEQ analysis (Fig. 6B).

In contrast to MHC-II–expressing TD antigen-specific IgG+ and IgM+ PC (Fig. 3F), steady-state IgM+ PCs presented across two phenotypically distinct compartments, with the major expression high surface levels of BTLA (B and T lymphocyte attenuator) with low to negligible MHC-II (Fig. 6C and SI Appendix, Fig. S5C). The BTLAhi IgM subset up-regulated a divergent set of transcriptional regulators (e.g., Ifi1, Ifi4, Rnu3, Crl2, Bcl2, Fosb, Afs6, Egr2, Smad3, and Fas) that contrasted significantly to the MHC-IIhi BTLAlo subset (e.g., E2f2, Rnu2, Notch 1, Notch2, Bhlha15, Mki67, Pena, Top2a, Ubr2c, Eaf2) (Fig. 6 D and E). These transcriptional differences extended to cell-surface molecules (e.g., Ly6d, Slamf9, Sla3a2, Tmem170b, and Ifnar2 in BTLAhi; Ly6c1, Ly75, Tnfrsf9, and Cd9 in BTLAlo), as well as intracellular and secreted cellular components (Ich1, Ichb1, and Ifng in BTLAhi; Mzb1, Edem1, Aurbk, Il22, and Il1a in BTLAlo). These differences demonstrate separable control over a wide spectrum of potential IgM+ PC effector functions for these two subsets, with further transcriptional divergence from recently formed antigen-specific effector IgM+ (SI Appendix, Fig. 5D).

IgG2b+ PC from the steady state shared many transcriptional features seen in the NP-specific IgG2b+ response (SI Appendix, Fig. S5E). However, significant IgG2b+ PC transcriptional differences were seen across all classes of cell function regulators (nuclear, cell membrane, intracellular, and secretory) when contrasted to naive B cells and the broad steady-state IgM+ PC programs (Fig. 6 F–I).

Distinct IgA PC Programming for Mucosal Immunity. As expected, due to their origins and mucosal targeting function, IgA-expressing PC are most abundant in the Peyer’s patch but can also be found in the BM and the spleen at steady-state (SI Appendix, Fig. S6). All Blimp-1+ IgA PCs expressed high levels of both intracellular and surface IgA (SI Appendix, Fig. S7A). Using the Blimp-1 reporter model, we isolated IgM+ class-switched IgA+ PC for gene-expression studies (Fig. 7A), which were broadly separated in UMAP clusters based on the full range of gene expression from qtSEQ analysis (Fig. 7B). In contrast to IgM+ PCs, the majority of the IgA+ PC cohort expressed intermediate to high levels of MHC-II and lower levels of BTLA (Fig. 7C and SI Appendix, Fig. S5C). However, IgA+ PC maintain high levels of the BCR coreceptor CD79b, akin to IgM+ PC and opposite to IgG2b+ PC (Fig. 7D). IgA+ expression of CD98 and PD-L1 was similar to IgM+ PCs but had the lowest expression of the BCR coreceptor CD19. Only IgA+ PCs expressed a substantial fraction of Ccr9, contrasted by Cxcr3 expression by IgM+ and IgG+ PCs (Fig. 7D and SI Appendix, Fig. S7B–D). As all of these protein-expression trends extended into the BM-derived population of IgA+ PCs, this indicated class conserved rather than location-driven programming (SI Appendix, Fig. S7E). Hence, even at the broad level of surface phenotype, it is clear that IgA+ PC express a different molecular program to PC of other antibody isotypes.

Comparing the IgA+ transcriptional program to naive B cells highlighted many of the canonical features of the PC program seen across all PC isotypes (decreased B cell identity: Pax5, Bach2, Spib, Bel6, decreased antigen processing and presentation: Cd74, H2-dma, H2-abb1; increased lineage transcription factors: Pdcd1, Ifi4, Pou2af1; increased Sdc1, Ly6c1, Cd44, Mzb1, Rec2) (Fig. 7E and SI Appendix, Fig. S7F). However, multiple molecular features were exaggerated in the steady-state IgA+ PC compartment in contrast to IgM+ and IgG2b+ PCs, such as the increased expression of major transcriptional regulators (Runx2, Klf13, Gata1, Junb), TGF-β responsive transcription factors (Klf4, Cited2), and UPR and secretion modifiers (Afα1, Xhp1, El2, If2r) (Fig. 7 F and G). That IgA+ PCs exhibited differential expression of chemokine receptors (Ccr7, Ccr9, Ccr10), the highest levels of growth factor receptors (Tnfrsf13b, Tnfrsf17), surface receptor sensors (Tmem176b, Lgals, Tbrs), and secretory environmental modifiers (Ifng, S100a9) indicates the capacity for enhanced chemotaxis, survival, and environmental sensing in the mucosal microenvironment over IgM+ and IgG2b+ PCs. Expression of these divergent phenotypic and transcriptional programs may uniquely and differentially impact IgA+ PC localization, survival, and effector functionality.

Discussion

In these studies, we targeted a single-cell RNA-seq platform connected surface phenotype with unique transcriptional programming of class-specific PCs. Divergent transcriptional programming defined early-forming IgM+ from type 1 IgG+ subclass PCs, which indicated different effector roles. By comparing effector and post-GC PC cohorts, we highlighted the acquisition of unique molecular programs resulting from Tfh cell-directed CSR and GC cycling necessary for memory-phase survival and function.
Here, we provide evidence that beyond the shared programs, different antibody isotype-specific PCs express divergent transcriptional components that indicate the quality of T cell help received before terminal differentiation. These differences imprint functional characteristics on the terminally differentiated PCs in an isotype-specific manner to influence intercellular communication, long-term post-GC cellular homing, and longevity. Understanding these basic differences will help to target and enhance varied immune effector outcomes. Extension into steady-state immunity supported the broad IgM+ and type 1 IgG+ PC transcriptional divergence seen in effector PCs revealed two transcriptionally distinct subpopulations within IgM+ PCs. Interrogation of type 3 IgA+ PCs revealed programmatic capacity for mucosal protective functions. Taken together, these separable immune response modules for PC transcriptional control emphasize unique class-specific molecular programming that shape

Fig. 5. Separable inflammatory post-GC PC programs. (A) Surface protein expression of MHC-II, Cxcr3, and Fas in antigen-specific day 5 and day 14 PCs compared to naive B cells. Numbers indicate percentage of cells expressing each marker (mean ± SEM, n = 6 to 9 mice, ****P < 0.0001, ns, not significant; two-tailed unpaired Student’s t-test). (B) UMAP reduction (see Materials and Methods for details) of antigen-specific day 5 and day 14 PCs using gene expression (n = 291 day 5 and n = 520 day 14 PCs). Based on the index-sorting data, the known cell identity was overlaid (day 5 PCs colored orange, and day 14 PCs blue). (C) Differences in gene expression plotted by statistical significance and presented as a volcano plot comparing day 5 (colored orange) and day 14 (blue) antigen-specific PCs (IgG2a+ and IgG2b+). Expression with a fold-change less than 0.5 log2 and P > 0.05 (negative binomial) are colored black. (D) The number of genes up-regulated aligning to the indicated GO pathway showing day 5 PCs in orange and day 14 PCs in blue. (Cell cycle, GO:0051726; negative adhesion, GO:0007162; positive adhesion, GO:0022409; cytokine sig pathway, GO 0019221; positive cytokine production, GO:0001819; negative apoptosis, GO:0043066; inflammatory response, GO:0006954; transcription, GO:0006355.) (E and F) Differences in gene expression plotted by statistical significance and presented as a volcano plot comparing day 5 (colored orange) and day 14 (colored blue) antigen-specific PCs for IgG2a+ (E) or IgG2b+ (F). Expression with a fold-change less than 0.5 log2 and P > 0.05 (negative binomial) are colored black. (G) Pseudobulk heatmap representation of differentially expressed genes and their location of function (nucleus, surface membrane, and cytosol and secreted) between day 5 and day 14 PCs split by subclass (IgG2a and IgG2b). Heatmaps show genes up in day 5 (Upper heatmap) and up in day 14 (Lower heatmap) antigen-specific PCs. The color scale is based on z-score distribution.
early effector responses and impact long-lived memory PC compartments.

Effector PCs are formed rapidly upon antigen exposure and are important for differentially impacting immune responses through class-specific antibody binding of Fc receptors (10–12). Effector IgM+ PCs up-regulated genes coding for Lbp3 and Tgfb2 that form a secreted complex, which can induce Th17 or regulatory T cell (T\textsubscript{REG}) formation in a concentration-dependent manner (51), suggesting effector IgM+ PCs may be capable of influencing a pro- or antiinflammatory environment. Additionally, IgM+ up-regulation of secreted costimulatory cytokine IL-1α may impact proinflammatory IL-1R-driven signaling. In line with their antibody-driven inflammatory function, IgG2a+ and IgG2b+ PCs each up-regulated additional proinflammatory molecules; however, both
subclasses also up-regulated antiinflammatory *Slpi*, known to prevent runaway proinflammatory activity (52). Therefore, we demonstrate that effector PCs are transcriptionally poised for separable class-linked functionality via additional secreted molecules.

Gene up-regulation of inhibitory cell-surface proteins (*Ctla4, Slamf7*) by IgM+ PCs would permit T cell modulation during T cell cognate contact, while IgG2a+ PC-elevated expression of *Tnfsf9* (CD137L), involved in bidirectional signaling after cognate contact, may direct increased inflammatory activity (53). As all effector PCs retained MHC-II expression, they may participate in T cell cognate interactions and induce additional TH cell functions through MHC-II antigen presentation, as we have previously demonstrated in class-switched post-GC PCs (40). Thus, each class of effector PC may be capable of impacting and influencing TFH cell functionality.

The GC reaction is critical for generating antigen-specific immunological memory through the formation of memory B cells. The GC reaction is critical for generating antigen-specific immunological memory through the formation of memory B cells.
cells and post-GC PCs poised for long-term functionality in specialized survival niches. During GC cycling, higher-affinity B cells receive increased T cell help (54, 55) and are more likely to exit as PCs (31–33, 56–58). Additional T cell interactions may be responsible for imprinting unique transcriptional programs in post-GC PCs. Similarly, late influenza antigen-specific PC transcriptional programs diverged across the major antibody isotypes (35). Distinctions in programs directing homing, metabolism, and effector function were seen in this model. Additionally, this infection also generated IgA PC responses with the greatest degree of transcriptional divergence and associated with very different homing characteristics. After transit to the BM, PCs exhibit transcriptional and phenotypic changes (29, 59–61). However, upon GC exit it is already apparent that post-GC PCs are broadly and indelibly changed to accommodate their memory role. For example, they lose cell cycle and division programs that allow entry into a quiescent state and negative regulators of apoptosis for long-term survival. Post-GC PCs up-regulated extrinsic signal receptors, costimulatory molecules, and cell-adhesion regulators. These effector molecules could contribute to enhanced T cell interaction during GC cycling and may be important for survival niche maintenance via exogenous secreted survival signals and extrinsic contact from support cells, such as TREGs (62).

The steady-state IgM\(^+\) PC compartment phenotypically split into two subsets defined by BTLA\(^+\)MHC-II\(^{lo}\) and BTLA\(^-\)MHC-II\(^{hi}\) expression. As mentioned earlier, subsets of CD138\(^+\) IgM\(^+\) PCs have been shown to secrete IL-10 and IL-35, which suppress effector CD4\(^+\) cells and innate cells to enact a regulatory B cell (B\(_{REC}\)) role (19, 63, 64). The BTLA\(^-\)MHC-II\(^{lo}\) IgM\(^+\) compartment identified here exhibited shared programs with regulatory IgM\(^+\) PCs, such as higher expression of Irf4 (19), down-regulation of cell-cycle-linked genes, reduced MHC-II protein, and expression of BLTA (associated with Bcl6 gene up-regulation in regulatory IgM\(^+\) PCs) (63). In contrast, the high protein expression of MHC-II by the second IgM\(^+\) subpopulation was more similar to help T cell-driven antigen-specific PCs and steady-state IgG\(^+\) PCs. This BTLA\(^-\)MHC-II\(^{lo}\) IgM\(^+\) compartment up-regulated antigen processing and presentation programs, T cell costimulatory proteins (e.g., Cd40lg, Tnf\(_i\)), and signal molecules, which may indicate capacity for modulating T\(_{H}\) cell function during MHC-II antigen presentation, as described previously (40). BCR repertoire analysis and functional studies with adoptive transfer will be needed to establish the stability and relatedness of these two subsets to each other and to known IgM PC compartments.

Thus, we propose that the phenotypically defined subsets represent transcriptionally distinct IgM\(^+\) PC cohorts: a regulatory compartment and a T cell-interacting compartment primed for antigen presentation that is TD in origin.

In the steady state, T cell-dependent gut-derived antigens generate type 3 IgA\(^+\) B cells that can enter GCs to undergo affinity maturation and exit as post-GC PCs (41, 65–68). Multiple features of this IgA splenic program are shared with IgA PCs resident in the small intestine lamina propria and BM compartments recently reported by Allman and colleagues (42). Expression of PD-L1 by IgA\(^+\) PCs may be retained from GC cycling where PD-1/PD-L1 interactions are critical for cell survival and post-GC PC formation (69). Additionally, PD-L1\(^+\)MHC-II\(^{hi}\) IgA\(^+\) PCs in the lamina propria induced FoxP3\(^+\) T\(_{REC}\) formation in the presence of TGF-\(\beta\) (70), which are important for maintaining T\(_{H}\)17 and IgA\(^+\) PC homeostasis (71). We detected IgA\(^+\) PC up-regulation of many complementary molecular programs with T\(_{H}\)17 cells, such as transcription factors (Klf4, Cited2, and surface protein expression [e.g., Tmem176b (72), Fas (73), and chemokine receptors Ccr7, Ccr9, and Cclr10 (74)]) that may confer similar mucosal defense functionality. Although T\(_{H}\)17 cells can induce formation of IgA\(^+\) PCs (75), up-regulation of Ifng by IgA\(^+\) PCs may in turn allow modulation of T\(_{H}\)17 formation and function through secretion of IFN-\(\gamma\) (76). These reciprocal molecular programs of T\(_{H}\)17 and IgA\(^+\) PCs may result from shared formation in a TGF-\(\beta\)-rich environment prior to IgA\(^+\) PC survival niche localization in the spleen (77, 78), similar to previous reports that gut IgA\(^+\) PC translocate to BM survival niches for systemic antibody secretion (79–81).

Previous studies on humans have identified preferential and sequential switching capacity of B cells (82) and identified class-linked heterogeneity, which may influence B cell fate and function (83–85). As each murine antibody class investigated in this study has a homologous isotype in humans, this work is applicable to ongoing human research, particularly for PC-driven diseases, immunotherapeutics, and vaccine design. Programmatic studies in multiple myeloma have led to approval of the anti-Slamf7 drug Elotuzumab (86) and proposals for Hif1a suppression as therapy (87); our studies identified up-regulation of Slamf7 in IgM\(^+\) PCs and Hif1a in IgG\(_{2a}\)\(^+\) PCs. Therefore, further exploration of the class-specific molecular programs in PCs can potentially identify targetable surface molecules or transcriptional pathways pertinent to PC-directed diseases.

Chimeric antigen receptor T cells marked the advent of synthetically engineered immunotherapeutics, which has now expanded into B cells (88–90). The specificities of known neutralizing antibodies introduced to BCR gene loci generated target-specific antibodies without prior antigen encounter, GC cycling, or affinity maturation after PC induction (88–90). Beyond specificity, these “synthetic” PCs need to function and survive long term in appropriate niches. Our studies detailing the molecular programming of post-GC memory-phase PCs provides elements of an isotype-specific transcriptional roadmap for future studies. Previous work has indicated B cell class may impact GC entry (84), or the terminal differentiation pathway into memory B or plasma cells (91). Herein, our study emphasizes that B cell class may impact immune function beyond Fc binding. We propose that each class of PCs is transcriptionally primed to differentially participate in and impact ongoing immune responses via ancillary effector functionality that must be considered during vaccine design.

Materials and Methods

Mice. C57BL/6 (B6), B6.CD45.1 (B6.SJL-PtprcaPepcb/BoyJ), and Blimp-1 YFP mice (provided by Susan Kaech, Salk Institute for Biological Studies, San Diego, CA) were bred and housed in specific pathogen-free conditions. All experiments were done in compliance with federal laws and institutional guidelines as approved by The Scripps Research Institutional Animal Care and Use Committee.

Data Availability. The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE205114) (92). All other study data are included in the main text and SI Appendix.

ACKNOWLEDGMENTS. This work was supported by NIH AI047231, AI040215, and AI071182 and Bill & Melinda Gates Foundation BMGF OPP1154835 (to M.G.M.-W.).

Author affiliations: Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA 92037.
1. M. McHeyzer-Williams, S. Oiktsu, N. Wang, L. McHeyzer-Williams, Molecular programming of B cell memory. Nat. Rev. Immunol. 12, 24-34 (2011).
2. S. Coty, T follicular helper cell differentiation, function, and roles in disease. Immunol. Rev. 216, 529-542 (2014).
3. C. M. Snapper, W. E. Paul, Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype- and tissue-specific gene expression. Science 236, 946-947 (1987).
4. S. G. Tangel, A. Ferguson, D. T. Avey, C. S. Ma, P. D. Hodgkin, Isotype switching by human B cells is division-associated and regulated by cytokines. J. Immunol. 169, 4298-4306 (2002).
5. J. A. Rico et al., Class-switch recombination occurs infrequently in germinal centres. Immunity 51, 310-320 (2019).
6. J. O. J. Sheas, W. E. Paul, Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 327, 1098-1102 (2010).
7. E. D. Vazquez et al., Intrahepatic lymphoid cells: 10 years on. Cell 174, 1054-1066 (2018).
8. R. J. Bunker et al., Class-switch programming of class-specific B cell memory by IL-7 and B/ΔRFx. Immunity 13, 604-611 (2000).
9. B. W. Higgins, L. J. McHeyzer-Williams, M. G. McHeyzer-Williams, Programming isotype-specific plasma cell function. Trends Immunol. 30, 345-357 (2019).
10. F. Nimmerjahn et al., FOXP3-deficiency results in FOXP3(−) plasmablast cell production in vivo. J. Exp. Med. 207, 305-309 (2011).
11. J. J. Oates et al., Single cell dissection of plasma cell heterogeneity in symptomatic and asymptomatic myeloma. Nat. Med. 24, 1867-1876 (2018).
12. W. Y. Lam et al., Metabolic and transcriptional modules independently diversify plasma cell function. Cell Rep. 29, 2479-2492 (2020).
13. M. G. McHeyzer-Williams, M. J. Mclanen, G. J. Nossal, P. A. Loral, The dynamics of T cell-dependent B cell responses in vivo. Immunity 9, 119-127 (1997).
14. M. G. McHeyzer-Williams, M. J. Mclanen, P. A. Lalcool, Antigen-driven B cell differentiation in vivo. J. Exp. Med. 185, 1701-1706 (1997).
15. L. J. McHeyzer-Williams, M. G. McHeyzer-Williams, Antigen-specific B cell memory. Expression and replenishment of a novel b220(−) memory B cell compartment. J. Exp. Med. 119, 1149-1166 (2000).
16. L. J. McHeyzer-Williams, M. G. McHeyzer-Williams, Developmentally distinct Th cells control plasma cell differentiation and RORgammat function. Nature 453, 236-240 (2008).
17. F. Y. Jin, C. Nathan, D. Radzioch, A. Ding, Secretory leukocyte protease inhibitor: A macrophage product induced by and anti-bacterial to pathogenic yersinia. Cell 88, 417-426 (1997).
18. B. K. Choi, H. W. Lee, The murine CD137/CD137 ligand-signalling: A Signal platform generating signal complexity. Front Immunol. 11, 553715 (2020).
19. Z. Shulman et al., Dynamic signaling by T follicular helper cells during germinal center B cell selection. Science 345, 1058-1062 (2014).
20. G. D. Vities et al., Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. Cell 143, 592-605 (2010).
21. A. D. Gillin et al., Independent roles of switching and hypermutation in the development and persistence of B lymphocyte memory. Immunity 44, 749-781 (2016).
22. P. Shimakawa et al., Regulation of germinal-center cells into the memory B cell compartment. Nat. Immunol. 17, 861-869 (2016).
23. N. J. Kratjer et al., Differentiation of germinal center B cells into plasma cells is initiated by high-affinity antigen and can be directed by Cib by T cells. J. Exp. Med. 214, 1259-1267 (2017).
24. H. C. Ruzanski et al., Sustained antigenic responsedepend on CD20 function in bone marrow-resident plasma cells. J. Exp. Med. 208, 1435-1446 (2011).
25. S. Chevrier et al., CD93 is required for maintenance of antibody secretion and persistence of plasma cells in the bone marrow niche. Proc. Natl. Acad. Sci. U.S.A. 106, 3895-3900 (2009).
26. J. W. Lam et al., Mitochondrial protein import promotes long-term survival of antibody-secreting plasma cells. Immunity 45, 60-73 (2016).
27. A. Galant-Marecky et al., T regulatory cells support plasma cell populations in the bone marrow. Cell Rep. 18, 1906-1917 (2016).
28. A. L. Kino et al., Jag-3-induced receptor expression identifies immunosuppressive natural regulatory plasma cells. Immunity 49, 120-133 e9 (2018).
29. E. C. Russer, C. Mauri, Regulatory B cells: Origin, phenotype, and function. Immunity 42, 607-612 (2015).
30. M. Kastamotou et al., The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Sci. Immunol. 3, 018–019 (2012).
31. B. Pierigret et al., Re-utilization of germinal centers in multiple Myer’s patches results in highly synchronized, oligoclonal, and affinity-matured IgA responses. Mucosal Immunol. 6, 122-135 (2013).
32. H. Chen et al., BCR selection and affinity maturation in Peyer’s patch germinal centres. Nat. Immunol. 14, 412-425 (2013).
33. A. Biam et al., BCR affinity differentially regulates colonization of the subepithelial dome and infiltration into germinal centers within Peyer’s patches. Nat. Immunol. 20, 482-492 (2019).
34. K. L. Good-Jacobson et al., PD-1 regulates germinal center cell survival and the formation and differentiation of long-lived plasma cells. J. Exp. Med. 216, 2159-2161 (2017).
35. T. l. Doi et al., IgA plasma cells express the negative regulatory co-stimulatory molecule programmed cell death 1 ligand and have a potential tolerogenic role in the intestine. Biochem. Biophys. Res. Commun. 425, 918-923 (2015).
36. C. Neumann et al., c-Maf-dependent T follicular helper cell control of intestinal T17 cells and IgA establishes host-microbiota homeostasis. Nat. Immunol. 20, 471-481 (2019).
37. L. Dujon et al., ROR gamma (α) T cells selectively express redox cation channels linked to the Galpy apparatus. Sci. Rep. 6, 23682 (2016).
38. G. Meyer duHorte et al., A lymphotoxin B promotes helper T cell development by binding and inqueering transcription factor STAT. Immunity 48, 556-569 (2017).
39. C. Wang, S. G. Kang, J. Lee, Z. Son, C. H. Kim, The roles of DCB in migration of Th17 cells and regulation of effector T-cell balance in the gut. Mucosal Immunol. 2, 173-183 (2009).
40. K. Hoto et al., Plasticity of Th17 cells in Peyer’s patches is responsible for the induction of T cell-dependent IgA responses. Nat. Immunol. 14, 372-379 (2013).
41. G. J. Martinez, R. I. Nurieva, X. O. Yang, C. Dong, Regulation and function of proinflammatory Th17 cells. Ann. N. Y. Acad. Sci. 1143, 186-211 (2008).
42. E. Jiang et al., Splenic long-lived plasma cells promote the development of follicular helper T cells from origins. Cell 170, 1882-1895 (2017).
43. J. T. L. Doi et al., Inflammatory signaling in T follicular helper cell-driven IgA responses depends on CD20 function in bone marrow plasma cells. Immunity 39, 2357-2368 (2018).
44. A. Lemke et al., Long-lived plasma cells are generated in mucosal immune responses and contribute to the bone marrow plasma cell pool in mice. Mucosal Immunol. 9, 83-97 (2016).
45. R. Lien et al., Strong clonal relationship between serum and gut IgA despite different plasma cell origins. Cell 20, 1237-1247 (2017).
46. F. Horne et al., Lineage-tracing of human B cells reveals the in vivo landscape of human antibody class switching via photoactivatable photoactivatable Ly108. Cell 170, 101064 (2021).
47. R. D. Glass et al., An integrated multi-omic single-cell atlas of human B cell identity. Immunity 53, 217-232 e5 (2020).
48. H. W. King et al., Single-cell analysis of human B cell maturation predicts how antibody class switching shapes selection dynamics. Sci. Immunol. 6, eabc6921 (2021).
85. D. Croote, S. Damianis, K. C. Nadeau, S. R. Quake, High-affinity allergen-specific human antibodies cloned from single IgE B cell transcriptomes. *Science* **362**, 1306–1309 (2018).

86. M. A. Dimopoulos et al., Elotuzumab plus pomalidomide and dexamethasone for multiple myeloma. *N. Engl. J. Med.* **379**, 1811–1822 (2018).

87. E. Borsi et al., Hypoxia inducible factor-1 alpha as a therapeutic target in multiple myeloma. *OncoTarget* **5**, 1779–1792 (2014).

88. T. C. Cheong, M. Compagno, R. Chiarle, Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system. *Nat. Commun.* **7**, 10934 (2016).

89. J. E. Voss et al., Reprogramming the antigen specificity of B cells using genome-editing technologies. *eLife* **8**, e42995 (2019).

90. V. Greiner et al., CRISPR-mediated editing of the B cell receptor in primary human B cells. *Science* **12**, 369–376 (2019).

91. K. Kometani et al., Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. *Immunity* **39**, 136–147 (2013).

92. B. W. Higgins et al., Isotype-specific plasma cells express divergent transcriptional programs. NCBI Gene Expression Omnibus (GEO). https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205114. Deposited 26 May 2022.