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Transcriptional analysis of the human IgE-expressing plasma cell differentiation pathway

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31 Abstract

32 IgE is secreted by plasma cells (PCs) and is central to allergic disease. Using an ex vivo 33 tonsil B cell culture system, which mimics the Th2 responses in vivo, we have recently 34 characterized the development pathway of human IgE-expressing PCs. In this system, 35 as in mice, we reported the predisposition of IgE-expressing B cells to differentiate into PCs. To gain a comprehensive understanding of the molecular events involved in the 36 37 differentiation of human IgE⁺ B cells into PCs we have used the Illumina HumanHT-38 12 v4 Expression BeadChip array to analyse the gene expression profile of ex vivo 39 generated human IgE⁺B cells at various stages of their differentiation into PCs. We also 40 compared the transcription profiles of IgE⁺ and IgG1⁺ cells to discover isotype-specific 41 patterns. Comparisons of IgE⁺ and IgG1⁺ cell transcriptional profiles revealed 42 molecular signatures specific for IgE⁺ cells, which diverge from their IgG1⁺ cell 43 counterparts upon differentiation into PCs. At the germinal center (GC) stage of 44 development, unlike in some mouse studies of IgE biology, we observed similar rates 45 of apoptosis and no significant differences in the expression of apoptosis-associated 46 genes between the IgE⁺ and IgG1⁺ B cells. We identified a gene interaction network 47 associated with early growth response 1 (EGR1) that, together with the up-regulated 48 IRF4, may account for the predisposition of IgE⁺ B cells to differentiate into PCs. 49 However, despite their swifter rates of PC differentiation, the transcription profile of 50 IgE⁺ PCs is more closely related to IgE⁺ and IgG1⁺ plasmablasts (PBs) than to IgG1⁺ 51 PCs, suggesting that the terminal differentiation of IgE⁺ cells is impeded. We also show that IgE⁺ PCs have increased levels of apoptosis suggesting that the IgE⁺ PCs generated 52 in our in vitro tonsil B cell cultures, as in mice, are short-lived. We identified gene 53 54 regulatory networks as well as cell cycle and apoptosis signatures that may explain the 55 diverging PC differentiation programme of these cells. Overall, our study provides a 56 detailed analysis of the transcriptional pathways underlying the differentiation of 57 human IgE-expressing B cells and points to molecular signatures that regulate IgE⁺ PC 58 differentiation and function.

59

60 Introduction

IgE plays a central role in the pathogenesis of allergic disease (1, 2). Although IgE is the least abundant antibody in the circulation, its binding to the high affinity IgE receptor (FccRI) on mast cells and basophils is critical for the manifestation of immediate hypersensitivity to allergens and allergic inflammation (1, 2). IgE is secreted by PCs, which represent the terminal stage of B cell differentiation, after immunoglobulin class switching to IgE in precursor B cells (3).

- 67 Important advances in understanding the regulation of IgE production have been made 68 over the last decade. The predisposition of IgE-switched cells to develop towards the 69 PC rather than the memory cell lineage is seen in both mouse and human systems (4-70 10). However, this could not be attributed to differences in the expression levels of the 71 PC differentiation master regulator, Blimp-1 (7, 9). Studies by IgE and IgG1 domain 72 swapping in mouse B cells show that membrane IgE (mIgE) signalling promotes 73 antigen-independent PC differentiation of IgE⁺ B cells (5, 10). The CH2-CH3 74 extracellular domains and the cytoplasmic tail contribute to this activity, but the key 75 component was the extracellular membrane-proximal domain (EMPD) (5, 10).
- The effect of mIgE signalling in PC differentiation has been suggested to involve IRF4 (5, 10), a transcription factor that regulates PC differentiation (11). However, we lack a more comprehensive knowledge of other molecular pathways that likely contribute to this process, especially in humans. Unlike in mouse, two isoforms of mIgE exist in humans, a short form (mIgEs), equivalent to the mouse mIgE, and a long form (mIgE_L) containing an EMPD that is 52 amino acids longer (12, 13). Expression of the mIgE_L by the human IgE⁺ B cells may also influence PC differentiation.

83 Using an ex vivo tonsil B cell culture system, stimulated with IL-4 and anti-CD40 in 84 *vitro* to generate IgE^+ cells, we have recently characterised the developmental pathway 85 of human IgE^+ and $IgG1^+$ PCs (7). In this system, we demonstrated that there are three 86 discrete stages of IgE⁺ PC development pathway, which we characterized phenotypically as IgE⁺ GC-like B cells (IgE^{lo}CD27⁻CD138⁻Bcl6^{hi}Pax5^{hi}Blimp1^{lo}), IgE⁺ 87 88 PC-like "PBs" (IgE^{hi}CD27⁺⁺CD138⁻Bcl6^{lo}Pax5^{lo}Blimp1^{hi}) and IgE⁺ PCs (IgE^{hi}CD27⁺⁺CD138⁺Bcl6^{lo}Pax5^{lo}Blimp1^{hi}) (7). A similar IgG1⁺ PC development 89 90 pathway was also observed. The IgE⁺ cells displayed cell cycle and proliferation rates 91 greater than their IgG1⁺ cell counterparts, and interestingly we also observed that the $\begin{array}{ll} 92 & \text{differentiation of IgE}^+ \text{B cells into PCs is accompanied by the modulation of mIgE}_L \text{ and} \\ 93 & \text{mIgE}_S \text{ surface expression (7). Here, to better understand the differentiation process of} \end{array}$

- 94 human IgE⁺ B cells into PCs and to identify key regulators of this process, we have used
- 95 the Illumina HumanHT-12 v4 Expression BeadChip array to define and compare the
- 96 transcriptomes of *ex vivo* generated IgE⁺ and IgG1⁺ B cells at various stages of their
- 97 differentiation into PCs.



98 Methods

99 Ethics

100 Tonsils were obtained from children undergoing routine tonsillectomies as a result of 101 tonsilities. Full written informed consent was given by parents or legal guardians of the 102 donors. The study was conducted at and in accordance with the recommendations of 103 King's College London and Guy's and St Thomas's NHS Fundation Trust and the 104 protocol was approved by the London Bridge Research Ethics Committee (REC 105 number 08/H0804/94).

106

107 Cell cultures

108 B cells were isolated from the dissected tonsil tissue on a density gradient (GE 109 Healthcare) followed by incubation with aminoethyl isothiouronium bromide-treated 110 sheep red blood cells to rosette T cells (TCS Biosciences). B cells were >95% CD19⁺ as determined by flow cytometric (FACS) analysis. Purified tonsil B cells were induced 111 112 to undergo class switching to IgE as previously (14). Briefly, 0.5×10^6 freshly purified 113 tonsil B cells were stimulated with IL-4 (200IU/ml; R&D Europe Systems Ltd) and 114 anti-CD40 antibody (0.5µg/ml; G28.5; American Type Culture Collection). After day 115 7 the population of IgG1⁺ and IgE⁺-switched cells gradually increased to a maximum 116 at 10 days when the cells were harvested for study.

117

118 FACS sorting of IgE⁺ and IgG1⁺ cells

119 Cultured cells were stained with a live/dead fixable stain dye (Life Technologies Ltd) 120 and anti-CD138 APC (Miltenyi Biotech) followed by fixation with 2% paraformaldehyde. Following washing with RNAsecure (Life Technologies Ltd) 121 122 treated PBS, supplemented with 100 U/mL of RNase inhibitor (Bioline Reagents Ltd) 123 and 5mM DL-dithiothreitol (Sigma-Aldrich Ltd), cells were permeabilised with 1% 124 molecular grade triton x100 (Sigma-Aldrich Ltd) containing 250U/mL of RiboSafe 125 RNase inhibitor and 5mM DL-dithiothreitol and intracellularly stained with anti-IgE 126 FITC (Vector Laboratories) and anti-IgG1 PE (Miltenyi Biotech) for 45 min on ice. The IgE¹°CD138⁻, IgE^{hi}CD138⁻ and IgE^{hi}CD138⁺ cells and their respective IgG1 127 128 counterparts were FACS sorted into melting buffer (Invitrogen) containing 1600U/mL 129 RiboSafe RNase inhibitors and 10mM DL-dithiothreitol and used for total RNA 130 extraction (see below).

- 131
- 132

133 RNA isolation

Total RNA was isolated using a previously described protocol (7) for the PureLink 134 135 FFPE total RNA isolation kit (Invitrogen). Briefly, cells were sorted into the melting 136 buffer containing 1600U/mL RNase inhibitor (Bioline) and 10mM DTT (Sigma-137 Aldrich Ltd) and stored at -80^oC before proceeding to the proteinase K treatment for 15 138 min at 60°C. Subsequently the manufacturers instructions were followed, including the 139 optional DNase digestion. The RNA was further cleaned using the RNeasy Mini Kit 140 RNA Cleanup protocol (Qiagen). RNA concentrations were measured using the 141 NanoDrop 2000 (Thermo Scientific) and RNA integrity assessed using the 2100 142 Bioanalyser instrument (Agilent Technologies, Inc).

143

144 Illumina BeadChips array

145 cDNA was synthesized and amplified from 40ng RNA using the Ovation Pico WTA 146 system V2 (NuGEN) and purified using the MiniElute Reaction Cleanup Kit (Qiagen). 147 Yield and purity were measured using the 2100 Bioanalyser instrument and the RNA 148 6000 Nano kit (Agilent). 4µg of amplified cDNA was biotin labeled with Encore Biotin 149 Module (NuGen), purified, concentrated and hybridized onto Illumina HumanHT-12 150 v4 Expression BeadChip array and scanned using the Illumina iScan platform. The 151 data was then subjected to QC analysis and normalization using Illumina's Genome 152 Studio Suite v1.0.

153

154 Microarray and gene network analysis

155 Assessment of differential gene expression and statistical analysis was performed in 156 Partek Genomics Suite 6.6. Unless otherwise stated 2 way ANNOVA analysis 157 (comparing donor identity and cell phenotype) was undertaken to 158 detect differential expression and the resultant gene lists were obtained by filtering 159 results by FDR < 0.05 and p value < 0.05 with fold changes > 1.5. The PANTHER 160 classification system (15) was used for the gene ontology (GO) analysis of the up-161 regulated and down-regulated genes. Unsupervised hierarchal clustering was 162 undertaken by K-means clustering of standardised gene intensity values, normalized so 163 that the mean is 0 and the standard deviation is 1 (z-score). Finally, gene regulatory 164 networks were investigated using Ingenuity Pathway analysis (IPA) (Qiagen 165 Bioinformatics) to identify known downstream targets of transcription factors (based 166 on Ingenuity knowledge database of mammalian interactions) or using Weighted Gene 167 Co-expression Network Analysis (WGCNA) analysis(16) to identify modules of highly 168 correlated genes. We related these modules to external sample traits using the169 eigengene network methodology (17).

- 170
- 171 The array data has been deposited in NCBI's Gene Expression Omnibus (18) and are172 accessible through GEO Series accession number GSE99948.
- 173

174 **RT-PCR**

175 RT-PCR was performed using TaqMan MGB gene expression assays and TaqMan 176 Universal PCR Master Mix on a Viia7 real-time PCR machine (Applied Biosystems). 177 Gene expression was normalized to an endogenous reference gene 18s rRNA 178 (Hs99999901 s1, Applied Biosystems). Off-the-shelf gene specific qPCR assays were 179 purchased from applied biosystems utilising Taqman MGB chemistry. All gene specific 180 assays were multiplexed with the 18s endogenous control assay and run in triplicate. 181 SDS software was used to determine relative quantification of the target cDNA 182 according to the $2^{-(\Delta\Delta ct)}$ method.

183

184 FACS analysis

To validate some of the differentially expressed genes we fixed, permeabilised and 185 186 stained cells as previously described(7). The antibodies used were as follows; anti-IL4R 187 APC (R&D), anti-CD27 FITC (Biolegend), anti-CD38 PE-CY7 (Biolegend), anti-188 CD20 FITC (Biolegend), anti-IRF4 alexa 647 (Invitrogen), anti-IRF8 APC 189 (Biolegend), anti-BLIMP1 APC (R&D) and anti-active Caspase 3 alexa 647 (BD 190 Biosciences). To determine the rates of apoptosis the IL-4 and anti-CD40 cultured cells 191 were harvested and the dead cells removed using the Easysep dead cell removal kit 192 (Stemcell). The cells were then recultured for 24h with IL-4 and anti-CD40, followed 193 by staining for Annexin V (eBioscience) and live/dead fixable violet dead stain kit (Life 194 Technologies). Data was collected on a BD FACSCanto (BD Biosciences) and events 195 were analyzed using FlowJo software version 10.4.2 (Tree Star).

196 197

198 **Results**

199 Transcriptional profile of GC and PC associated genes along the differentiation 200 pathway of IgE⁺ and IgG1⁺ cells

In order to determine the transcriptional profile of IgE⁺ and IgG1⁺ PCs, and their prescursors, after 10 days of culture with IL-4 and anti-CD40, tonsil B cells were sorted by flow cytometry into IgE⁺ and IgG1⁺ GC-like B cells, PC-like PBs and PCs (Figure 1A). Total RNA from the purified cells was isolated reverse transcribed, amplified and biotin labelled prior to transcriptional profiling using the Illumina HumanHT-12 v4 Expression BeadChip array.

207

208 To confirm and extend our phenotypic characterization of the IgE^+ and $IgG1^+$ PCs, and 209 their prescursors, we compared the transcriptional profile of known regulators and 210 markers of B cell differentiation into PCs (19-25) (Figure 1B). Genes previously 211 associated with GC reactions were highly expressed in both IgE⁺ and IgG1⁺ GC B cells 212 compared to IgE⁺ and IgG1⁺ PBs and PCs (e.g. *IL-4R* >3-fold, *STAT6* >2-fold, *AICDA* 213 >4-fold, BCL6 >3-fold). In contrast, genes associated with PC differentiation and 214 functions were highly expressed in both PBs and PCs compared to IgE⁺ and IgG1⁺ GC 215 B cells (e.g. IRF4 >3.5-fold, PRDM1 >4-fold, XBP1 >4-fold). The differential 216 expression of some of the genes was also confirmed at the protein level by flow 217 cytometery (Figure 1C). Overall, the data shows that our previously characterised cell 218 populations displayed a uniform profile with respect to these GC- and PC-associated 219 markers, consistent with the designated phenotype of the populations.

220 Distinct gene expression patterns at different stages of B cell differentiation into221 PCs

222 To determine the gene expression changes during the differentiation of GC B cells into 223 PCs, irrespective of Ig isotype, we performed a 2 way ANOVA, based on donor identity 224 and cell phenotype, yielding 726 annotated genes that were differentially expressed by 225 >1.5-fold (P-value of < 0.05, and FDR < 0.05) between any of the cell types. To identify 226 genes with distinct expression profiles across the three cell types we generated self-227 organising maps (SOMs) and identified 6 different patterns of gene expression 228 associated with either negative or positive regulation as cells differentiated into PCs 229 (Figure 2A and Suplementary Data 1).

230 GO analysis of the clustered genes revealed that cluster 1, identifying genes which 231 peaked at the PB stage, contained genes that were associated with type I interferon 232 signalling pathway (GO:0060337, fold enrichment = 27.74), such as *IRF4*, required for 233 PC differentiation (11), and IRE1-mediated unfolded protein responses (GO:0036498, 234 fold enrichment = 21.85), which activates XBP1 (26). Cluster 2 genes, which peaked at 235 the PC stage, are involved in co-translational protein targeting to membranes 236 (GO:0006613, fold enrichment = 13.75), endoplasmic reticulum to cytosol transport (GO:1903513, fold enrichment = 59.58), and endoplasmic reticulum unfolded protein 237 238 responses (GO:0030968, fold enrichment = 25.53). Examples include *PRDM1*, the well-known regulator of PC differentiation (27), and XBP1, which plays a key role in 239 240 protein folding, secretion and degradation (28). Expression of genes within cluster 3 241 also peaked at the PC stage. These genes were involved mainly in protein N-linked 242 glycosylation via asparagine (GO:0018279, fold enrichment = 26.99) and ER-243 associated ubiquitin-dependent protein catabolic process (GO:0030433, fold 244 enrichment = 15.64).

245 In contrast to clusters 1-3, genes within clusters 4-6 were down-regulated as B cells 246 differentiated into PCs. Consistent with the phenotype of cells, these clusters contained 247 genes previously shown to play an important role in establishing, maintaining or 248 mediating GC reactions (19, 20, 24), including IL4R (cluster 4), AICDA, FAS, IRF8 249 (cluster 5), BCL6 and CIITA (cluster 6). The main biological processes enriched within 250 cluster 4 are the cellular response to cytokine (GO:0034097, fold enrichment=4.59) and 251 the regulation of immune responses (GO:0050776, fold enrichment = 4.02). Genes 252 within cluster 5, primarily restricted to GC cells, were associated with various aspects 253 of cell division, including DNA unwinding involved in DNA replication (GO:0006268, 254 fold enrichment = 88.3), cell cycle phase transition (GO:0044770, fold enrichment = 255 7.78) and DNA replication (GO:0006260, fold enrichment = 11.88). Genes within 256 cluster 6, repressed particularly in PB cells, are associated with mitotic cell cycle phase 257 transition (GO:0044772, fold enrichment = 6.34) and lymphocyte activation 258 (GO:0046649, fold enrichment = 4.93).

Since these clusters contain genes with highly correlated expression profiles, we also
investigated whether they were known to be regulated by common transcription factors.
GO analysis of transcription factor binding sites (TFBS) revealed that all 6 clusters
were enriched for certain transcription factor binding sites (TFBS) (Table 1), either

specifically enriched in certain clusters (e.g. ETS2 and NFAT in cluster 1; PAX4 in
cluster 3; NFY and FOXO4 in cluster 4; E12, PU1 and E2F in cluster 6) or in more than
one cluster (e.g. SP1 and LEF1).

266 Next, to highlight the transcriptional changes during the PC differentiation of IgE⁺ and 267 IgG1⁺ cells, we constructed a series of Venn analysis diagrams using genes 268 differentially expressed (>1.5-fold change with a P-value of < 0.05, FDR < 0.05) along 269 their differentiation pathway into PCs (Figure 2B, C). The comparison showed that both 270 IgE⁺ PBs and IgE⁺ PCs shared a core of differentially up-regulated (351) and down-271 regulated (260) genes compared to IgE⁺ GC B cells, but also genes that distinguished 272 IgE⁺ PCs (96 up-regulated and 124 down-regulated) from PBs (77 up-regulated and 32 273 down-regulated) (Figure 2B and Supplementary Data 2). By comparison, while IgG1⁺ PBs and IgG1⁺ PCs also shared a core of differentially up-regulated (322) and down-274 275 regulated (407) genes compared to IgG1⁺ GC B cells, the number of differentially 276 expressed genes unique to IgG1⁺ PCs (213 up-regulated and 357 down-regulated) more 277 than doubled in comparison to that of IgE⁺PCs whereas those of IgG1⁺ PBs were almost 278 unchanged (72 up-regulated and 43 down-regulated) (Figure 2C and Supplementary 279 Data 2). The GO analysis of these genes show that the main biological processes 280 enriched with genes that are either up-regulated or down-regulated in IgE⁺ and IgG1⁺ 281 GC B cells, compared to their more differentiated cell populations, are consistent with 282 their phenotype (Supplementary Data 2).

The transcriptional profiles of IgE⁺ and IgG1⁺ cells diverge as PC differentiation proceeds

285 We have previously shown that IgE⁺ and IgG1⁺ cells display different biological 286 properties with regards to their differentiation potential (7). Upon examining the 287 expression levels of IRF4, which has been reported to be involved in the PC differentiation of mouse IgE⁺ GC B cells (10), we observed a significantly higher 288 289 expression of this transcription factor in IgE⁺ cells at the GC stage compared to their 290 IgG1⁺ cell counterparts (Figure 3A). To better understand the molecular pathways 291 underlying these biological differences we carried out a 2-way ANOVA analysis 292 comparing the genes unique to each IgE⁺ and IgG1⁺ cell differentiation stage. As 293 illustrated by the Venn analysis diagrams, IgE⁺ GC B cells share a similar pattern of 294 gene expression with the IgG1⁺ GC B cells (1532 similarly expressed genes), with only 295 7 up-regulated and 25 down-regulated genes in IgE⁺ GC B cells (Figure 3B and

Supplementary Data 3). At the PB stage of differentiation, IgE^+ cells had 940 unchanged, 26 down-regulated and 35 up-regulated genes compared to $IgG1^+$ cells. However, at the PC stage, IgE^+ and IgG^+ cells diverge in their transcriptional profiles and display a more distinctly different profile with 1125 unchanged, 164 downregulated and 255 upregulated genes in IgE^+ PCs compared to $IgG1^+$ PCs (Figure 3B and Supplementary Data 3).

To emphasise these diverging transcriptional profiles we subjected genes, the expression of which differed by >1.5 fold across any cell type, to hierarchal clustering (Figure 3C). Clustering confirmed that IgE^+ and IgG^+ GC cells were most similar. However, while $IgG1^+$ PCs have a very distinct transcriptional profile, IgE^+ PCs are more closely related to IgE^+ and $IgG1^+$ PBs. This observation was especially surprising, considering that we and others have previously shown that IgE^+ cells are more prone to differentiation than $IgG1^+$ cells (4, 7, 9).

309 To explore the origins of IgE⁺ and IgG1⁺ cell differences, we undertook a gene 310 regulatory network (GRN) analysis using the curated knowledge database in IPA, as well as a data-driven approach using WGCNA (16). IPA analysis on the differentially 311 312 expressed genes between IgE⁺ and IgG1⁺ GC-like B cells identified a gene interaction 313 network associated with the inducible zinc finger transcription factors, EGR1 and EGR2 314 (Figure 4A). The RT-PCR analysis confirmed the up-regulated EGR1 and EGR2 315 expression in IgE⁺ GC-like B cells (Figure 4B). These transcription factors are known 316 regulators of a number of genes and include those that are down-regulated (CASP3, 317 MYB, LDLR, GNAS, FTL, CCR2, CCND2, and NDRG1) or up-regulated (CAV1, FAS, 318 CD19, G3BP1, LOX5AP, NFKB1, MYBL1, TNF, TP53, SOD1) in IgE⁺ and IgG1⁺ GC 319 B cells compared to their more differentiated cell counterparts. This network also 320 contained genes upregulated (NCL, FCER2, CDC20, CCL3L3, and CCR1) or down-321 regulated (PTPN1, GADD45A, GADD45B, TIMP1, NDRG1, and RB1) in IgE⁺ PCs 322 compared to $IgG1^+$ PCs (Figure 4A).

In addition, WGCNA identified a co-expression network, which is enriched in IgE⁺ PCs (p = 0.003), containing a large number of ribosomal components and the differentially expressed transcriptional regulator *TCS22D3* and guanine exchange factor (*FAM116B*) (Figure 4C, 4D and Supplementary Data 4). 327 Overall these data suggest that the IgE⁺ and IgG1⁺ cells adopt an increasingly different 328 gene expression profile as they differentiate into PCs. The data also provide molecular

- 329 signatures that may account for some of the differences seen in the later stages of IgE^+
- and $IgG1^+$ cell differentiation.

331 Proliferative and apoptotic associated genes differentially expressed in IgE⁺ and

- 332 IgG1⁺ cells
- According to the GO analysis, among the most enriched biological processes associated with genes over-expressed in IgE⁺ PCs, compared to IgG1⁺ PCs, were *translation initiation* (GO:0006413, fold enrichment = 12.74), *mitotic cell cycle phase transition* (GO:0044772, fold enrichment = 6.09) and *mitotic cellular division* (GO:0007067, fold enrichment = 4.67), suggesting that IgE⁺ PCs are still cycling (Supplementary Data 3). These observations are consistent with our previously reported data (7), which show that the proliferative and cycling capacity of IgE⁺ PB and PCs is greater than that of their IsC1[±] cell counterparts
- 340 their $IgG1^+$ cell counterparts.

341 There are several differentially expressed genes that correlate with the enhanced 342 proliferation of IgE⁺ cells relative to their IgG1⁺ cell counterparts (Figure 5A). Among 343 these genes, RB1, an important regulator of the G1 checkpoint (29), and GADD45A, a 344 regulator of the G2-M checkpoint (30, 31), are upregulated in IgG1⁺ PBs and PCs, but 345 not in IgE⁺ PBs and PCs, when compared to GC B cells (Figure 5A). Other negative 346 regulators of the cell cycle progression up-regulated in IgG1⁺ PBs and PCs include 347 CDKN2B, HUS1 and E4F1. Conversely, we observe that IgE⁺ PBs and PCs, unlike 348 their IgG1⁺ cell counterparts, up-regulate the expression of a number of genes 349 associated with positive regulation of the cell cycle e.g. CDC25B, MYC, CSK1B, 350 FOXM1, CDCA3, AURKB, PLK4, CDC20, E2F2 (Figure 5A).

351 Contrary to recent reports suggesting that IgE⁺ GC B cells undergo increased apoptosis 352 compared to $IgG1^+$ GC B cells (5, 6), the expression of apoptosis-associated genes in 353 $IgG1^+$ and IgE^+ GC B cells is similar (Figure 5B). The exceptions are the pro-apoptotic 354 regulators BNIP3, BNIP3L and HKR, which are increased in IgG1⁺ GC cells, and 355 DAPK2, increased in IgE⁺GC cells. However, Annexin V and dead cell staining of the 356 cells after 24h of culture, reveales that IgE⁺ and IgG1⁺GC B cells have similar rates of 357 apoptosis (Figure 6A). This is also supported by their similar levels of activated 358 caspase-3 at day 10 of the culture with IL-4 and anti-CD40 (Figure 6B), suggesting that

359 unlike in the mouse system these cells undergo apoptosis at a similar rate. In contrast, 360 despite increased levels of TNFRSF13B (TACI) and TNFRSF17 (BCMA), two important contributors of PC survival (32, 33), in IgE⁺ PBs and PCs (Supplementary 361 362 Data 3), their rates of apoptosis and their expression levels of active caspase-3 are 363 increased compared to their IgG1⁺ cell counterparts (Figure 6A and 6B). We find that the expression of a number of apoptosis-associated genes was either up-regulated (e.g. 364 BNIP2, CASP3, FADD and MAP3K5) or down-regulated (e.g. DAPK2, BNIP3, 365 366 BNIP3L, BCL2L1 and CASP1) in both IgE⁺ PBs and PCs compared to their IgG1⁺ cell 367 counterparts (Figure 5B). In addition, BAG1, TP53INP1 and TP73 were downregulated and BCL2L11, CASP10 and TNFRSF25 were up-regulated only in IgE⁺ PCs 368 369 (Figure 5B). The differential expression of BCL2L1 and BCL2L11, which encode two well characterised regulators of apotosis, Bcl-xL and Bim, respectively, in IgE⁺ and 370 IgG1⁺ PCs was also confirmed by RT-PCR (Figure 6C). 371

372 Overall the data suggest that the apoptotic potential of IgE⁺ cells increases as they

373 differentiate into PCs and that IgE⁺ PCs may be inhibited from exiting the cell cycle, a

374 process that is required for the completion of the PC differentiation program (21, 22,

375 34).

376 **Discussion**

377 A notable feature of IgE^+ B cell development is the predisposition of IgE^+ GC B cells 378 to differentiate into PCs (6, 7, 9). In this study, we sought to obtain a better 379 understanding of the IgE^+ PC differentiation process by analysing gene expression in 380 human B cells at discrete stages of PC differentiation. We also compared IgE^+ and 381 $IgG1^+$ B cells to discover isotype-specific patterns.

382

We identified distinct gene expression patterns at different stages of B cell differentiation into PCs and found that at each stage both IgE^+ and $IgG1^+$ cells have distinct molecular signatures with well-characterised genes of B cell function and differentiation as well as other genes of unknown function. The analysis of genes recognised as critical for either the GC reaction or PC differentiation and function confirmed the phenotype of our previously characterised IgE^+ and $IgG1^+$ cells (7).

389

390 A previous study reported that the vast majority of mouse IgE^+ GC B cells undergo 391 apoptosis, owing to low mIgE expression and the resulting weak BCR signalling (6). 392 Thus, the canonical B cell differentiation programme is not observed. It was proposed 393 that IgE BCR directly promotes the apoptosis of IgE⁺ B cells (5, 35). However, the 394 evidence for this is conflicting, and our results are more consistent with another study 395 in the mouse, which also demonstrated similar rates of apoptosis in IgE^+ and $IgG1^+$ GC 396 B cells (10). Shedding further light on this matter, recent work has revealed that the 397 expression of the ε heavy chain itself on GC B cells leads to PC differentiation 398 uncoupled from antigen activity (5, 10). This antigen-independent PC differentiation 399 mediated by the IgE BCR involved IRF4. The increased levels of IRF4 expression in 400 our in vitro generated IgE⁺ GC-like B cells may also account, in part, for the accelerated 401 PC differentiation of human IgE⁺ B cells. Using the curated knowledge database in IPA, 402 we have identified two other transcriptional regulators, EGR1 and EGR2, that can 403 contribute to this process. EGR1 has been reported to regulate PC differentiation of B 404 cells(36) and EGR2 to be associated with T cell differentiation (37, 38). In future 405 studies it would be interesting to determine the mechanisms by which the different 406 expression levels of these transcription factors affect the differentiation rates of IgE⁺ 407 and $IgG1^+ B$ cells.

408 A novel finding of our study is that as IgE^+ and $IgG1^+$ B cells differentiate into PCs 409 their transcriptional profiles diverge, with IgE^+ and $IgG1^+$ PCs showing the greatest 410 difference. Consistent with our previously reported results (7), we observed that a 411 number of genes involved in the regulation of the cell cycle are differentially expressed 412 in IgE⁺ cells. For example, the protein product of RB1, which is repressed in IgE⁺ PBs 413 and PCs, can block the S-phase entry and growth by binding to the E2F1 transcription 414 factors and inhibiting its activity (29). Similarly, GADD45A, which can arrest the cell 415 cycle at the G2-M checkpoint by suppressing the CDC2/Cyclin B kinase activity (30, 416 31), is also down-regulated in IgE⁺ PBs and PCs. In contrast, *CDC25B* and *MYC*, two 417 positive regulators of the cell cycle and proliferation (39, 40), are both expressed at 418 elevated levels in the IgE⁺ cells.

In addition, using a WGCNA approach, we identified a large number of ribosomal proteins enriched in IgE⁺ PCs. It is known that the rate of translation is finely tuned to match cell proliferation (41, 42), and therefore increased ribosomal protein expression in the IgE⁺ PCs, compared to IgG1⁺ PCs, may be a consequence (or driver) of increased proliferation in these cells. Together these differences (and their downstream effects) may account for the maintenance of proliferative capacity as the IgE⁺ B cells differentiate into PCs.

426 Intriguingly, the analysis of the gene expression data revealed that the transcriptional 427 profile of IgE⁺ PCs was more closely related to that of IgE⁺ and IgG1⁺ PBs than to 428 IgG1⁺ PCs. It might be that the failure of IgE⁺ PCs to fully exit the cell cycle hinders 429 their completion of the PC differentiation programme. Additionally, discrepancies 430 between the IgE⁺ and IgG1⁺ PC transcriptional profiles might also be due to the up-431 regulation of the human $mIgE_{S}(7)$ on becoming PCs, which distinguishes these cells 432 from non-IgE⁺ PCs that down-regulate their mIg receptors as they become more 433 dedicated to antibody secretion.

434 As seen in the mouse (10), the increased rates of apoptosis suggests that the IgE⁺ PCs 435 generated in our tonsil B cell cultures may be short-lived PCs, which could account for 436 some of the transcriptional differences between IgE⁺ and IgG1⁺ PCs. Similarly, a recent 437 study, published during the review of our manuscript, reaffirmed the immature 438 transcriptional program and relatively poor survival capacity of differentiated IgE⁺ cells 439 isolated from the blood of peanut allergic patients (43). In support of this, our data show 440 that IgE⁺ PCs down-regulate BCL2L1 (Bcl-xL), which prevents apoptosis during the 441 PC differentiation by sequestring Bim (44), a pro-apototic protein encoded by 442 BCL2L11(45, 46), which is up-regulated in IgE⁺ PCs. Other pro-apoptotic associated 443 genes that are up-regulated in IgE^+ PCs, and which could account for their higher rates of apoptosis, include FADD (fas associated death domain) and MAP3K5 (47-49). 444 445 However, despite their increased rates of apoptosis, IgE⁺ PCs were expressing 446 significantely higher levels of TNFRSF13B and TNFRSF17, which encode two very 447 important regulators of PC survival, the transmembrane activator and CAML interactor 448 (TACI) and the B cell maturation antigen (BCMA), respectively (32, 33, 50, 51). The 449 differential expressions of pro- and anti-apoptotic associated genes suggests that IgE⁺ 450 and IgG1⁺ PCs may have different survival requirments, possibly related to the 451 microenvironment in which they reside(52, 53). This is highlighted by the serum IgE 452 titres and the IgE-mediated responses after immunosuppressive treatments that do not 453 affect the long-lived PCs (54-57), demonstrating the presence of long-lived IgE⁺ PCs. 454 Further work is needed to test the predicted effects of the cell cycling and apoptosis-455 associated genes on IgE⁺ PC differentiation and survival.

456

457 In summary, we have defined the molecular signature of the human IgE⁺ and IgG1⁺ cell 458 differentiation into PCs. We show that the transcriptional profile of IgE⁺ and IgG1⁺ 459 cells diverges as these cells differentiate into PCs. At the GC stage of development, we 460 observe similar rates of apoptosis between IgE⁺ and IgG1⁺ cells. However, IgE⁺ B cells 461 have increased levels of IRF4 and EGR1 which may predispose these cells into PC 462 differentiation. Significantly, IgE⁺ PCs have an immature gene expression profile that 463 is more related to IgE⁺ and IgG1⁺ PBs than to IgG1⁺ PCs. They continue cycling and exhibit increased rates of apoptosis. Overall, our data furthers our understanding of the 464 465 molecular events involved in the regulation of PC differentiation of IgE⁺ B cells and 466 the longevity of the generated IgE⁺ PCs.

467

468	Abbreviat	previations			
469	AID	Activation-induced cytidine deaminase			
470	EMPD	Extra-membrane proximal domain			
471	FDR	False discovery rates			
472	GC	Germinal Center			
473	GO	Gene ontology			
474	GRN	Gene regulatory network			
475	IPA	Ingenuity Pathway Analysis			
476	$mIgE_L$	Long form of membrane IgE			
477	mIgEs	Short form of membrane IgE			
478	PB	Plasmablast			
479	PC	Plasma cell			
480	SOM	Self-organising map			
481	WGCNA	Weighted gene co-expression network analysis			

482 **Conflict-of-interest**

- 483 The authors declare that they have no conflicts of interests.
- 484

485 Author Contributions

F.R. designed and performed experiments, analysed data and wrote the paper. H.B.,
performed experiments and analysed data. H.J.G. designed experiments, analysed data
and wrote the paper. D.J.F designed and performed experiments, analysed data and
wrote the paper. All authors reviewed the final manuscript.

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References

Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* (2008)
 8(3):205-17. doi: 10.1038/nri2273. PubMed PMID: 18301424.

Dullaers M, De Bruyne R, Ramadani F, Gould HJ, Gevaert P, Lambrecht BN.
The who, where, and when of IgE in allergic airway disease. *J Allergy Clin Immunol* (2012) 129(3):635-45. doi: 10.1016/j.jaci.2011.10.029. PubMed PMID: 22168998.

Song S. Gould HJ, Ramadani F. IgE responses in mouse and man and the persistence of
IgE memory. *Trends Immunol* (2015) 36(1):40-8. doi: 10.1016/j.it.2014.11.002.
PubMed PMID: 25499855.

512 4. Erazo A, Kutchukhidze N, Leung M, Christ AP, Urban JF, Jr., Curotto de
513 Lafaille MA, et al. Unique maturation program of the IgE response in vivo. *Immunity*514 (2007) 26(2):191-203. doi: 10.1016/j.immuni.2006.12.006. PubMed PMID: 17292640;
515 PubMed Central PMCID: PMCPMC1892589.

516 5. Haniuda K, Fukao S, Kodama T, Hasegawa H, Kitamura D. Autonomous
517 membrane IgE signaling prevents IgE-memory formation. *Nat Immunol* (2016)
518 17(9):1109-17. doi: 10.1038/ni.3508. PubMed PMID: 27428827.

6. He JS, Meyer-Hermann M, Xiangying D, Zuan LY, Jones LA, Ramakrishna L,
et al. The distinctive germinal center phase of IgE+ B lymphocytes limits their
contribution to the classical memory response. *J Exp Med* (2013) 210(12):2755-71. doi:
10.1084/jem.20131539. PubMed PMID: 24218137; PubMed Central PMCID:
PMCPMC3832920.

7. Ramadani F, Bowen H, Upton N, Hobson PS, Chan YC, Chen JB, et al.
Ontogeny of human IgE-expressing B cells and plasma cells. *Allergy* (2017) 72(1):6676. doi: 10.1111/all.12911. PubMed PMID: 27061189; PubMed Central PMCID:
PMCPMC5107308.

528 8. Talay O, Yan D, Brightbill HD, Straney EE, Zhou M, Ladi E, et al. IgE(+)
529 memory B cells and plasma cells generated through a germinal-center pathway. *Nat*530 *Immunol* (2012) 13(4):396-404. doi: 10.1038/ni.2256. PubMed PMID: 22366892.

531 9. Yang Z, Sullivan BM, Allen CD. Fluorescent in vivo detection reveals that
532 IgE(+) B cells are restrained by an intrinsic cell fate predisposition. *Immunity* (2012)
533 36(5):857-72. doi: 10.1016/j.immuni.2012.02.009. PubMed PMID: 22406270.

10. Yang Z, Robinson MJ, Chen X, Smith GA, Taunton J, Liu W, et al. Regulation
of B cell fate by chronic activity of the IgE B cell receptor. *Elife* (2016) 5. doi:
10.7554/eLife.21238. PubMed PMID: 27935477; PubMed Central PMCID:
PMCPMC5207771.

538 11. Ochiai K, Maienschein-Cline M, Simonetti G, Chen J, Rosenthal R, Brink R, et
539 al. Transcriptional regulation of germinal center B and plasma cell fates by dynamical
540 control of IRF4. *Immunity* (2013) 38(5):918-29. doi: 10.1016/j.immuni.2013.04.009.
541 PubMed PMID: 23684984; PubMed Central PMCID: PMCPMC3690549.

542 12. Peng C, Davis FM, Sun LK, Liou RS, Kim YW, Chang TW. A new isoform of
543 human membrane-bound IgE. *J Immunol* (1992) 148(1):129-36. PubMed PMID:
544 1727861.

545 13. Zhang K, Saxon A, Max EE. Two unusual forms of human immunoglobulin E
546 encoded by alternative RNA splicing of epsilon heavy chain membrane exons. *J Exp*547 *Med* (1992) 176(1):233-43. PubMed PMID: 1613458; PubMed Central PMCID:
548 PMCPMC2119292.

Ramadani F, Upton N, Hobson P, Chan YC, Mzinza D, Bowen H, et al. Intrinsic
properties of germinal center-derived B cells promote their enhanced class switching to
IgE. *Allergy* (2015) 70(10):1269-77. doi: 10.1111/all.12679. PubMed PMID:

552 26109279; PubMed Central PMCID: PMCPMC4744720.

- Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-scale gene function
 analysis with the PANTHER classification system. *Nat Protoc* (2013) 8(8):1551-66.
 doi: 10.1038/nprot.2013.092. PubMed PMID: 23868073.
- Langfelder P, Horvath S. WGCNA: an R package for weighted correlation
 network analysis. *BMC Bioinformatics* (2008) 9:559. doi: 10.1186/1471-2105-9-559.
 PubMed PMID: 19114008; PubMed Central PMCID: PMCPMC2631488.

Langfelder P, Horvath S. Eigengene networks for studying the relationships
between co-expression modules. *BMC Syst Biol* (2007) 1:54. doi: 10.1186/1752-05091-54. PubMed PMID: 18031580; PubMed Central PMCID: PMCPMC2267703.

562 18. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene 563 expression and hybridization array data repository. *Nucleic Acids Res* (2002) 564 30(1):207-10. PubMed PMID: 11752295; PubMed Central PMCID: PMCPMC99122.

565 19. Corcoran LM, Tarlinton DM. Regulation of germinal center responses, memory
566 B cells and plasma cell formation-an update. *Curr Opin Immunol* (2016) 39:59-67. doi:
567 10.1016/j.coi.2015.12.008. PubMed PMID: 26799208.

568 20. De Silva NS, Klein U. Dynamics of B cells in germinal centres. *Nat Rev*569 *Immunol* (2015) 15(3):137-48. doi: 10.1038/nri3804. PubMed PMID: 25656706;
570 PubMed Central PMCID: PMCPMC4399774.

571 21. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. *Nat Rev*572 *Immunol* (2005) 5(3):230-42. doi: 10.1038/nri1572. PubMed PMID: 15738953.

- 573 22. Cocco M, Stephenson S, Care MA, Newton D, Barnes NA, Davison A, et al. In
 574 vitro generation of long-lived human plasma cells. *J Immunol* (2012) 189(12):5773-85.
 575 doi: 10.4049/jimmunol.1103720. PubMed PMID: 23162129.
- Jourdan M, Caraux A, De Vos J, Fiol G, Larroque M, Cognot C, et al. An in
 vitro model of differentiation of memory B cells into plasmablasts and plasma cells
 including detailed phenotypic and molecular characterization. *Blood* (2009)
 114(25):5173-81. doi: 10.1182/blood-2009-07-235960. PubMed PMID: 19846886;
 PubMed Central PMCID: PMCPMC2834398.
- 581 24. Klein U, Tu Y, Stolovitzky GA, Keller JL, Haddad J, Jr., Miljkovic V, et al.
 582 Transcriptional analysis of the B cell germinal center reaction. *Proc Natl Acad Sci U S*583 A (2003) 100(5):2639-44. doi: 10.1073/pnas.0437996100. PubMed PMID: 12604779;
 584 PubMed Central PMCID: PMCPMC151393.
- 585 25. Shi W, Liao Y, Willis SN, Taubenheim N, Inouye M, Tarlinton DM, et al.
 586 Transcriptional profiling of mouse B cell terminal differentiation defines a signature
 587 for antibody-secreting plasma cells. *Nat Immunol* (2015) 16(6):663-73. doi:
 588 10.1038/ni.3154. PubMed PMID: 25894659.
- 589 26. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced 590 by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active 591 transcription factor. *Cell* (2001) 107(7):881-91. PubMed PMID: 11779464.
- 59227.Martins G, Calame K. Regulation and functions of Blimp-1 in T and B593lymphocytes.AnnuRevImmunol(2008)26:133-69.doi:59410.1146/annurev.immunol.26.021607.090241.PubMed PMID: 18370921.
- Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, Qian SB, Zhao H, et al.
 XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles,
 and increases protein synthesis in plasma cell differentiation. *Immunity* (2004)
 21(1):81-93. doi: 10.1016/j.immuni.2004.06.010. PubMed PMID: 15345222.
- 599 29. Henley SA, Dick FA. The retinoblastoma family of proteins and their regulatory
 600 functions in the mammalian cell division cycle. *Cell Div* (2012) 7(1):10. doi:
 601 10.1186/1747-1028-7-10. PubMed PMID: 22417103; PubMed Central PMCID:
 602 PMCPMC3325851.

- 603 30. Wang XW, Zhan Q, Coursen JD, Khan MA, Kontny HU, Yu L, et al. GADD45 induction of a G2/M cell cycle checkpoint. Proc Natl Acad Sci U S A (1999) 604 605 96(7):3706-11. PubMed PMID: 10097101; PubMed Central PMCID: PMCPMC22358. Zhan Q, Antinore MJ, Wang XW, Carrier F, Smith ML, Harris CC, et al. 606 31. 607 Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-608 regulated protein Gadd45. Oncogene (1999) 18(18):2892-900. doi: 609 10.1038/sj.onc.1202667. PubMed PMID: 10362260.
- Moreaux J, Hose D, Jourdan M, Reme T, Hundemer M, Moos M, et al. TACI
 expression is associated with a mature bone marrow plasma cell signature and C-MAF
 overexpression in human myeloma cell lines. *Haematologica* (2007) 92(6):803-11.
 Epub 2007/06/07. PubMed PMID: 17550853; PubMed Central PMCID:
 PMCPMC2789280.
- 615 33. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et
 616 al. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J Exp*617 *Med* (2004) 199(1):91-8. Epub 2004/01/07. doi: 10.1084/jem.20031330. PubMed
 618 PMID: 14707116; PubMed Central PMCID: PMCPMC1887725.
- 619 34. Care MA, Stephenson SJ, Barnes NA, Fan I, Zougman A, El-Sherbiny YM, et
 620 al. Network Analysis Identifies Proinflammatory Plasma Cell Polarization for Secretion
 621 of ISG15 in Human Autoimmunity. *J Immunol* (2016) 197(4):1447-59. doi:
 622 10.4049/jimmunol.1600624. PubMed PMID: 27357150; PubMed Central PMCID:
 623 PMCPMC4974491.
- 5. Laffleur B, Duchez S, Tarte K, Denis-Lagache N, Peron S, Carrion C, et al.
 Self-Restrained B Cells Arise following Membrane IgE Expression. *Cell Rep* (2015).
 doi: 10.1016/j.celrep.2015.01.023. PubMed PMID: 25683713.
- 627 36. Oh YK, Jang E, Paik DJ, Youn J. Early Growth Response-1 Plays a Non628 redundant Role in the Differentiation of B Cells into Plasma Cells. *Immune Netw* (2015)
 629 15(3):161-6. doi: 10.4110/in.2015.15.3.161. PubMed PMID: 26140048; PubMed
 630 Central PMCID: PMCPMC4486779.
- 631 37. Du N, Kwon H, Li P, West EE, Oh J, Liao W, et al. EGR2 is critical for
 632 peripheral naive T-cell differentiation and the T-cell response to influenza. *Proc Natl*633 *Acad Sci U S A* (2014) 111(46):16484-9. doi: 10.1073/pnas.1417215111. PubMed
 634 PMID: 25368162; PubMed Central PMCID: PMCPMC4246296.
- 635 38. Ogbe A, Miao T, Symonds AL, Omodho B, Singh R, Bhullar P, et al. Early
 636 Growth Response Genes 2 and 3 Regulate the Expression of Bcl6 and Differentiation
 637 of T Follicular Helper Cells. *J Biol Chem* (2015) 290(33):20455-65. doi:
 638 10.1074/jbc.M114.634816. PubMed PMID: 25979336; PubMed Central PMCID:
 639 PMCPMC4536451.
- 64039.Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25641phosphatases.Curr Opin Cell Biol (2006) 18(2):185-91.64210.1016/j.ceb.2006.02.003.PubMed PMID: 16488126.
- 643 40. Bretones G, Delgado MD, Leon J. Myc and cell cycle control. *Biochim Biophys*644 *Acta* (2015) 1849(5):506-16. doi: 10.1016/j.bbagrm.2014.03.013. PubMed PMID:
 645 24704206.
- 646 41. Donati G, Montanaro L, Derenzini M. Ribosome biogenesis and control of cell
 647 proliferation: p53 is not alone. *Cancer Res* (2012) 72(7):1602-7. doi: 10.1158/0008648 5472.CAN-11-3992. PubMed PMID: 22282659.
- 649 42. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? *Nat Rev Cancer*650 (2003) 3(3):179-92. doi: 10.1038/nrc1015. PubMed PMID: 12612653.
- 651 43. Croote D, Darmanis S, Nadeau KC, Quake SR. High-affinity allergen-specific 652 human antibodies cloned from single IgE B cell transcriptomes. *Science* (2018)
- 653 362(6420):1306-9. Epub 2018/12/14. doi: 10.1126/science.aau2599. PubMed PMID: 30545888

655 44. Gaudette BT, Iwakoshi NN, Boise LH. Bcl-xL protein protects from C/EBP homologous protein (CHOP)-dependent apoptosis during plasma cell differentiation. J 656 289(34):23629-40. 657 Biol Chem (2014)Epub 2014/07/16. doi: 10.1074/jbc.M114.569376. PubMed PMID: 25023286; PubMed Central PMCID: 658 659 PMCPMC4156059.

660 45. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, et al.
661 Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte
662 homeostasis, and to preclude autoimmunity. *Science* (1999) 286(5445):1735-8. Epub
663 1999/11/27. PubMed PMID: 10576740.

46. Woess C, Tuzlak S, Labi V, Drach M, Bertele D, Schneider P, et al. Combined
loss of the BH3-only proteins Bim and Bmf restores B-cell development and function
in TACI-Ig transgenic mice. *Cell Death Differ* (2015) 22(9):1477-88. Epub 2015/02/24.
doi: 10.1038/cdd.2015.8. PubMed PMID: 25698446; PubMed Central PMCID:
PMCPMC4532784.

47. Lin FR, Huang SY, Hung KH, Su ST, Chung CH, Matsuzawa A, et al. ASK1
promotes apoptosis of normal and malignant plasma cells. *Blood* (2012) 120(5):103947. doi: 10.1182/blood-2011-12-399808. PubMed PMID: 22723553.

48. Ranjan K, Pathak C. FADD regulates NF-kappaB activation and promotes
ubiquitination of cFLIPL to induce apoptosis. *Sci Rep* (2016) 6:22787. Epub
2016/03/15. doi: 10.1038/srep22787. PubMed PMID: 26972597; PubMed Central
PMCID: PMCPMC4789601.

49. Ranjan K, Surolia A, Pathak C. Apoptotic potential of Fas-associated death
domain on regulation of cell death regulatory protein cFLIP and death receptor
mediated apoptosis in HEK 293T cells. *J Cell Commun Signal* (2012) 6(3):155-68.
Epub 2012/07/14. doi: 10.1007/s12079-012-0166-2. PubMed PMID: 22791313;
PubMed Central PMCID: PMCPMC3421020.

50. Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam KP, et al. Cutting edge:
the dependence of plasma cells and independence of memory B cells on BAFF and
APRIL. *J Immunol* (2008) 180(6):3655-9. Epub 2008/03/07. PubMed PMID:
18322170.

685 51. Ou X, Xu S, Lam KP. Deficiency in TNFRSF13B (TACI) expands T-follicular
686 helper and germinal center B cells via increased ICOS-ligand expression but impairs
687 plasma cell survival. *Proc Natl Acad Sci U S A* (2012) 109(38):15401-6. Epub
688 2012/09/06. doi: 10.1073/pnas.1200386109. PubMed PMID: 22949644; PubMed
689 Central PMCID: PMCPMC3458353.

690 Smurthwaite L, Walker SN, Wilson DR, Birch DS, Merrett TG, Durham SR, et 52. 691 al. Persistent IgE synthesis in the nasal mucosa of hay fever patients. Eur J Immunol 692 31(12):3422-31. Epub 2001/12/18. doi: 10.1002/1521-(2001)693 4141(200112)31:12<3422::AID-IMMU3422>3.0.CO;2-T. PubMed PMID: 694 11745361.

53. Luger EO, Fokuhl V, Wegmann M, Abram M, Tillack K, Achatz G, et al.
Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. J *Allergy Clin Immunol* (2009) 124(4):819-26 e4. Epub 2009/10/10. doi:
10.1016/j.jaci.2009.06.047. PubMed PMID: 19815119.

699 54. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et
700 al. Competence and competition: the challenge of becoming a long-lived plasma cell.
701 *Nat Rev Immunol* (2006) 6(10):741-50. doi: 10.1038/nri1886. PubMed PMID:
702 16977339.

55. Brunette MG, Bonny Y, Spigelblatt L, Barrette G. Long-term
immunosuppressive treatment of a child with Takayasu's arteritis and high IgE
immunoglobulins. *Pediatr Nephrol* (1996) 10(1):67-9. PubMed PMID: 8611360.

- 56. Wyczolkowska J, Brzezinska-Blaszczyk E, Maslinski C. Kinetics of specific
 IgE antibody and total IgE responses in mice: the effect of immunosuppressive
 treatment. *Int Arch Allergy Appl Immunol* (1983) 72(1):16-21. PubMed PMID:
 6603423.
- 710 57. Holt PG, Sedgwick JD, O'Leary C, Krska K, Leivers S. Long-lived IgE- and
- 711 IgG-secreting cells in rodents manifesting persistent antibody responses. Cell Immunol
- 712 (1984) 89(2):281-9. PubMed PMID: 6542454.



713 **Table legend**

714 **Table 1. Summary of temporal clusters.**

- 715 The top GO biological processes with the lowest P-value and a fold enrichment
- 716 threshold of > 10 are shown. TFBS significant at 5% threshold and known to regulate
- 717 > 10 genes are shown. For a list of genes related to each of the clusters see
- 718 Supplementary Data 1. (ns: Not significant)



719 Figure legends

Figure 1. Expression profile of GC and PC associated genes in sorted IgE⁺ and IgG1⁺ cell populations.

722 A, IL-4 and anti-CD40 stimulated tonsil B cells were harvested on day 10 of the culture 723 and surface stained for CD138, intracelluar IgE and IgG1 and FACS sorted into GC B cells (IgE^{lo} CD138⁻ and IgG1^{lo} CD138⁻), PBs (IgE^{hi} CD138⁻ and IgG1^{hi} CD138⁻), and 724 PCs (IgE^{hi} CD138⁺ and IgG1^{hi} CD138⁺). **B**, Heatmap of GC and PC associated genes in 725 726 each of the sorted IgE^+ and $IgG1^+$ cell populations. Each column represents the gene 727 expression profiles of the different phenotypic cell populations sorted from four 728 different tonsil B cell cultures. C, Flow cytometric validation of seven differentially 729 expressed genes in IgE^+ and $IgG1^+$ cell populations. Data are representative of 6 730 experiments.

Figure 2. Distinct gene expression patterns and identification of genes unique different stages of B cell differentiation into PCs.

733 A, Clustering of genes differentially expressed along the differentiation pathway of B 734 cells into PCs regardless of the Ig isoform was undertaken by the production of 735 unsupervised Self-organising Maps (SOM). B, Venn diagrams showing overlaps and 736 differences between genes that were significantly (p <0.05) up-regulated or down-737 regulated by > 1.5 fold in IgE⁺ cells along their differentiation pathway into PCs. C, 738 Venn diagrams showing overlaps and differences between genes that were significantly 739 (p <0.05) up-regulated or down-regulated by > 1.5 fold in IgG1⁺ cells along their 740 differentiation pathway into PCs.

Figure 3. The relationship between the IgE⁺ and IgG1⁺ cells along their differentiation pathway.

743 A, Expression levels of IRF4 in IgE^+ and $IgG1^+$ cells as determine by flow cytometery. 744 Data show the fold change in median flurosence intensity (MFI) of anti-IRF4 stained 745 cells relative to $IgG1^+$ GC-like B cells (n = 6). Statistical analysis was performed using 746 the One-Way ANOVA, Dunnett's test (*P < 0.05). **B**, Visualisation of gene expression 747 differences between IgE⁺ and IgG1⁺ cells along their PC differentiation pathway. Genes 748 differentially expressed (>1.5-fold, p<0.05) at each IgE^+ and $IgG1^+$ cell differentiation 749 stage underwent a 2-way ANNOVA analysis. The number of genes that were 750 significantly (p <0.05) up-regulated or down-regulated by > 1.5 fold in IgE⁺ cells 751 compared to IgG1⁺ cells at GC, PB and PC are highlighted by the Venn diagrams. C, 752 Unsupervised K-means heirarchical clustering of all genes differentially expressed in

- 753 IgE⁺ and IgG1⁺ cells along their differentiation pathway. Each column represents the
- 754 mean gene expression profile from all four donors of the specified phenotypic group.

Figure 4. Identification of gene interaction and co-expression networks associated with IgE⁺ PC differentiation.

A, IPA was performed on genes that were differentially expressed between IgE⁺ and 757 758 IgG1⁺ cells (>1.5-fold and P <0.05). The gene network was identified based on the 759 literature contained in the IPA knowledge database. Target genes of the EGR1 and 760 EGR2, shown in the figure, were found to be differentially expressed by more than 1.5-761 fold (P < 0.05) either in IgE⁺ and IgG1⁺ GC-like B cells compared to PBs or PCs or in 762 IgE⁺ cells compared to IgG1⁺ cells along their PC differentiation pathway. **B**, RT-PCR 763 validation of EGR1 and EGR2 expression in IgE⁺ and IgG1⁺ GC-like B cells. Data 764 represent the mean +/- SD of the relative quantification (RQ). Statistical analysis was 765 performed using the t test with Welch's correction (*P < 0.05, **P < 0.01). C, 766 Identification of a module of highely correlated genes, by WGCNA analysis encoding 767 a large number of ribosomal proteins, that is enriched in IgE⁺ PCs. In total this network 768 contains 547 genes, however, to improve network visibility only those with a weight 769 above 0.075 are shown. This de-novo co-expression network was negatively correlated 770 with the IgG1⁺ PCs (correlation coefficient -0.65, p=0.003). The genes up-regulated 771 (red) or down-regulated (blue) by more than 1.3-fold in IgE⁺ PCs compared to IgG1⁺ 772 PCs, whereas genes with less than 1.3-fold difference are shown as uncoloured. The 773 shape of each node reflects the biological function of each gene, as determined by GO 774 analysis. More detailed information about the top candidate genes displayed in the 775 network can be found in the Supplementary Data 4. D, RT-PCR validation of RPL31, 776 which is up-regulated, and TSC23D3 that is down-regulated in IgE⁺ and IgG1⁺ PCs. 777 Data represent the mean +/- SD of the relative quantification (RO). Statistical analysis 778 was performed using the unpaired t test with Welch's correction (*P < 0.05, **P <779 0.01).

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783 Figure 5. Cell cycle/proliferation-associated genes differentially expressed in IgE⁺

784 and IgG1⁺ cells.

A, Heatmap of cell cycle/proliferation–associated and **B**, pro- and anti-apoptotic genes differentially expressed along the PC differentiation pathway of both IgE^+ and $IgG1^+$ cells, and differentially expressed in IgE^+ cells compared to $IgG1^+$ cells. Each column in the heat maps shown represents the mean gene expression profile from all four donors of the specified phenotypic group.

790

791 Figure 6. IgE⁺ PCs have increased rates of apoptosis compared to IgG1⁺ PCs

792 A, After 24h of reculture with IL-4 and anti-CD40, the IgE⁺ and IgG1⁺ cells were 793 stained with annexin V and a live/dead fixable dye. The lower left quadrant within each 794 dot plot (negative for Annexin V and live/dead stain) corresponds to the viable cells 795 and the data shown are representative of three different experiments. **B**, On day 10 of 796 the culture, the activity of Caspase 3 was determined by staining with anti-active 797 Caspase 3 antibody. The data show the fold change in MFI of active Caspase 3 within 798 each IgG1⁺ cell population made relative to their respective IgE⁺ cell counterparts. 799 Statistical analysis was performed using the one way ANNOVA test with Bonferroni 800 correction (*P < 0.05). C, RT-PCR validation of *BCL2L11* and *BCL2L1* expression in IgE⁺ and IgG1⁺ PCs. Data represent the mean +/- SD of the relative quantification (RQ). 801 802 Statistical analysis was performed using the unpaired t test with Welch's correction (*P< 0.05, **P < 0.01). 803

Table 1			
	N T / N	Top GO biological process	
Cluster	Notable genes	(fold enrichment >10, p <e-05)< th=""><th>TFBS >10 genes</th></e-05)<>	TFBS >10 genes
1	MCL1, IRF4	Type I interferon signaling	ETS2 (11 genes p<0.0012),
		pathway, endoplasmic reticulum	AP4 (12 genes p<0.0019),
		unfolded protein response,	SP1 (17 genes p<0.0036),
		cellular response to unfolded	NFAT (12 genes p<0.0088)
		protein	
2	CD27,	Protein exit from endoplasmic	SP1 (14 genes p<0.0026),
	PRDM2,	reticulum	LEF1 (13 genes p<0.0039)
	IRF1, XBP1		
3	CD38,	Protein N-linked glycosylation	SP1 (25 genes p<2.3e-5), LEF1
	CD79A	via asparagine	(21 genes p<0.00026), MYC
			(11 genes p<0.0006), PAX4
			(11 genes p<0.0038)
4	BCLIIA,	NS	MAZ (17 genes $p<0.0001$),
	CD19, IL4R		NFY (12 genes $p<0.00012$),
			AP4 (12 genes $p<0.0005$), EOXO4 (12 genes $p<0.0011$)
			FOA04 (15 genes p<0.0011), SP1 (15 genes p<0.0027)
5		DNA replication	SP1 (17 genes $p<0.0027$) SP1 (17 genes $p<0.0026$)
5	CCL 17	Divitiepheation	MAZ (11 genes $p < 0.00020$),
	CCL22 FAS		LEF1 (12 genes $p < 0.0152$)
	IRF8 MVR		E12 (11 genes p<0.0153)
6	RATE3	Mitosis	E_{2}^{2} E2E (11 genes p<7 3e-9) SP1
U	BCI 6	WIROSIS	(33 genes $p < 7.2e-9$). ETS (19
	CD79B		genes $p < 2.2e07$), LEF1 (28
	CD83 SPIR		genes p<1.4e-6), E12 (26
	CD05, 51 ID		genes p<1.5e-6), MYC (15
			genes p<1.3e-5), PU1 (12
			genes p<1.2e-5)

Figure 1.JPEG











5B





0.0

1.8

Figure 6.JPEG





