Transcriptional Signatures of Influenza A/H1N1-specific IgG Memory-like B Cell Response in Older Individuals

Iana H. Haralambieva[1], **Inna G. Ovsyannikova**[1], **Richard B. Kennedy**[1], **Michael T. Zimmermann**[2], **Diane E. Grill**[2], **Ann L. Oberg**[2], and **Gregory A. Poland**[1,*]

[1]Mayo Clinic Vaccine Research Group

[2]Division of Biomedical Statistics and Informatics, Department of Health Science Research, Mayo Clinic, Rochester, MN 55905 USA

Abstract

Background—Studies suggest that the recall-based humoral immune responses to influenza A/H1N1 originates from activated memory B cells. The aim of this study was to identify baseline, early and late blood transcriptional signatures (in peripheral blood mononuclear cells/PBMCs) associated with memory B cell response following influenza vaccination.

Methods—We used pre- and post-vaccination mRNA-Seq transcriptional profiling on samples from 159 subjects (50–74 years old) following receipt of seasonal trivalent influenza vaccine containing the A/California/7/2009/H1N1-like virus, and penalized regression modeling to identify associations with influenza A/H1N1-specific memory B cell ELISPOT response after vaccination.

Results—Genesets and genes (p-value range 7.92E-08 to 0.00018, q-value range 0.00019 to 0.039) demonstrating significant associations (of gene expression levels) with memory B cell response suggest the importance of metabolic (cholesterol and lipid metabolism-related), cell migration/adhesion, MAP kinase, NF-kB cell signaling (chemokine/cytokine signaling) and

transcriptional regulation gene signatures in the development of memory B cell response after influenza vaccination.

Conclusion—Through an unbiased transcriptome-wide profiling approach, our study identified signatures of memory B cell response following influenza vaccination, highlighting the underappreciated role of metabolic changes (among the other immune function-related events) in the regulation of influenza vaccine-induced immune memory.

Keywords

Influenza; Human; Influenza Vaccines; Vaccination; Influenza A Virus; H1N1 Subtype; Enzyme-Linked Immunospot Assay; Sequence Analysis; RNA; Gene ExpressionB-Lymphocytes; Aging; Immunity; Humoral; Immunity

Introduction

Influenza and influenza-related complications pose a major health risk for vulnerable populations, such as the elderly. Despite an increasing annual influenza vaccine coverage rate, influenza-related morbidity and mortality increase with age. This is primarily due to poor immune response to influenza vaccine and increased susceptibility to influenza infection among older persons [1–5].

Immunosenescence is a profound age-related dysregulation of the immune system, which leads to impaired immunity/protection following immunization, or significant morbidity and mortality from infection as a result of age-associated changes in both innate and adaptive immune system components [6, 7]. While individual factors are beginning to be elucidated, there is a lack of a comprehensive, systems-level understanding of how age affects adaptive immunity and immune memory due to vaccination, particularly in regard to influenza response [2, 8–13]. Several recent studies focused their efforts on identification of immune signatures and biomarkers associated with influenza-specific antibody (mostly hemagglutination-inhibition/HAI) titers as the currently accepted correlate of protection from influenza illness [9–13]. Data from the literature point to the predominantly recall nature of the influenza A/H1N1-specific humoral immune responses, with IgG-producing influenza vaccine-specific plasmablasts originating from activated memory B cells specific for conserved (in several strains) influenza virus epitopes [14, 15]. Thus, influenza-specific memory B cells (MBCs) comprise an important pool of cells capable of responding to stimulation by differentiation into plasma cells to mount a rapid secondary immune response to pathogenic challenge and contribute to both short-term and long-term immunity. To date, no biological markers/models exist that explain and/or predict the development of influenza-specific memory B cell immune response after vaccination.

The aim of the current study is to identify baseline, early and late transcriptional signatures (in peripheral blood mononuclear cells/PBMCs) associated with influenza A/H1N1-specific MBC response following receipt of seasonal trivalent inactivated (influenza A/H1N1-containing) influenza vaccine in an older (50–74 years old) study population.

Methods

The methods described herein are similar or identical to those published for our previous studies [1, 16–21].

Study Subjects

Details on study recruitment and study cohort characteristics have been provided elsewhere [1, 16–18]. As previously reported, the study population consisted of 159 subjects (50–74 years old), enrolled at Mayo Clinic, Rochester, MN, following receipt of the 2010–2011 seasonal trivalent influenza vaccine, containing the A/California/7/2009 H1N1-like, A/Perth/16/2009 H3N2-like, and B/Brisbane/60/2008-like viral strain antigens [18]. Blood samples for this study were collected prior to vaccination (baseline) and at two timepoints post-vaccination (Day 3 and Day 28), as shown in Fig. 1 (study design). All study participants provided written informed consent, and all study procedures were approved by Mayo Clinic's Institutional Review Board.

B cell ELISPOT assay

We quantified influenza virus-positive B cells (memory-like IgG B cells) at all timepoints pre- and post-vaccination in subjects' PBMCs using the Mabtech ELIspot^{PLUS} kit for human IgG (Mebtech Inc.; Cincinnati, OH), according to the manufacturer's specifications and as previously described [1, 16]. The results are presented as spot-forming units (SFUs) per 2×10^5 cells as subjects' medians (median of influenza virus-specific response, measured in quadruplicate). Intra-class correlation coefficients, assessing the correlation between replicate measurements in this assay at baseline, were high (0.88) [1, 16].

Library Preparation and Next Generation Sequencing (NGS)

The mRNA NGS methods are similar or identical to those published for our previous transcriptomics studies [19–21]. In brief, libraries were prepared from total RNA extracted from PBMCs (without additional viral/antigen stimulation; all timepoints pre- and post-vaccination) and, after QA/QC validation, were loaded for single-end read sequencing on the Illumin HiSeq 2000 (Illumina; San Diego, CA) using the Illumina's Single Read Cluster Generation kit (v2), and the 50 Cycle Illumina Sequncing Kit (v3). The sequencing reads were aligned to the human genome build 37.1 using TopHat (1.3.3) and Bowtie (0.12.7). HTSeq (0.5.3p3) was used to perform gene counting, while BEDTools (2.7.1) was used to count the reads mapping to individual exons [22–24].

Statistical methods

Here, we provide an overview of statistical and bioinformatic methods used; full details are available as supplemental information. All assays were run utilizing randomized block designs, keeping all samples for a subject adjacent in run order [25–27]. Data quality was assessed graphically and via variance criteria. Our analytical strategy consisted of two complementary approaches. The biology-to-gene approach is a deductive knowledge-driven approach in which analyses are performed at the pathway or network-module level defined externally to our data [28]. The gene-to-biology approach is an inductive, data-driven approach in which analyses are performed at the gene level, or on gene modules defined

from our data. Data-filtering strategies were used to reduce the chance of false positives. Penalized-regression methods were used to identify key genes or genesets associated with B cell ELISPOT response to vaccination [29, 30]. The dependent variable was the change in influenza virus-specific B cell ELISPOT from Day 0 to Day 28. Network-modeling strategies were used to integrate and interpret results from statistical models. Prioritized genesets were integrated using network biology resources (HPRD [31], CCSB [32], PID [33], and the 7.8% of STRING [34] with confidence score 70%). Networks were visualized using Cytoscape [35] version 3.2.1 and layouts refined using AllegroLayout v. 2.2.1 [36]. The biologic functions of genesets were evaluated using GO term [37, 38] and 138 KEGG canonical pathways [39, 40] manually curated to remove disease-associated and derived pathways (see Supplemental Information for more details).

Results

Subjects Demographics and Memory B cell ELISPOT response

The demographic and immune response variables have been previously described by us [1, 16, 18]. A total of 159 subjects (98.7% Caucasians) participated in this study. Females represented 61.6% of the cohort. The median (IQR) age of the cohort was 59.5 (55.3, 66.3) years. One participant was excluded due to the cDNA library failing quality control (QC) metrics, leaving a total of 158 subjects for analysis.

The dynamics of the influenza A/H1N1-specific memory B cell ELISPOT response after vaccination and the variation of this immune outcome in our study subjects and its correlation with other immune response variables have been previously described in detail [1, 16]. In summary, we observed a significant increase (from 11 SFUs per 2×10^5 PBMCs [IQR 5, 22] at baseline to 38 SFUs [IQR 18, 60] at Day 28, p-value=1.1×10^{-21}) of influenza-specific memory B cell frequencies post-vaccination, with 40.1% of the subjects demonstrating a considerable (4-fold) increase in influenza A/H1N1-specific B cell ELISPOT response (Day 0 compared to Day 28), which parallels the classical HAI definition of vaccine response [1, 16]. Details on the transcriptional responses in our study cohort have been previously published [41].

Linear models of B cell ELISPOT response using gene-to-biology approach

To assess the influence of transcriptomic changes on the memory B cell response in our cohort, we estimated per-variable association of baseline, early and late transcriptional changes with peak memory B cell ELISPOT response (Day 28-Day 0) (Table 1). The top 30 genes whose expression levels were significantly associated with memory B cell response at baseline (p-value range 7.59E-06 to 0.00012, q-value=0.027), early (p-value range 7.58E-06 to 0.00018, q-value=0.039), and late (p-value range 7.92E-08 to 1.958E-06, q-value range 0.00019 to 0.00032) timepoints after influenza vaccination are shown in Table 1. Information on all significant genes is included in Supplementary Table S1. Regression modeling results of B cell ELISPOT response, using data-driven transcriptional modules (gene-to-biology approach), are shown in Supplemental Information Fig. S1

Regression models of B cell ELISPOT response using biology-to-gene approach

We used externally defined pathways/genesets with significant (early [Day 3 – Day 0] and late [Day 28 – Day 0]) mRNA expression changes over time (p <0.005, n = 142) in cross-validated penalized regression models of memory B cell ELISPOT response (Day 28 – Day 0) after vaccination. The pathways and biological functions associated with memory B cell response are summarized in Table 2; for early (Day 3 – Day 0) gene expression, changes are centered around genes related to cholesterol/sterol biosynthesis and membrane function (MSE range 2.007 to 2.036, Table 2, Fig. 2A and -2B). Biological pathways for late (Day 28 – Day 0) gene expression changes, which are associated with memory B cell response (MSE range 1.98 to 2.029, Table 2), are related to genes functioning in cell adhesion and cell migration (Fig. 2C); cell signaling, including MAP kinase activity (Fig. 2D) and cytokine/ chemokine signaling (Fig. 2E); and transcriptional regulation (including regulation of key metabolism-related genes) (Fig. 1F).

Network modeling

Using the top nine statistically prioritized genesets/pathways (adjusted r^2 0.08), we identified three functional modules consisting of metabolic, epigenetic regulation, and cytokine/chemokine signaling. These modules are visually evident from a network layout that considers known gene-gene connectivity from PPI and pathway resources (force-directed; see Fig. 3).

Enrichment levels of canonical pathways and GO terms were tested (Supplemental Information Fig. S2). Many of the statistically prioritized genes are members of canonical signaling pathways that carry out important events in immune response. To strengthen the biological interpretation and validate our findings, we also investigated the effect of statistical significance threshold choice on pathway enrichment levels. As the inclusion threshold (geneset q-value) was lowered to include between the top 9 and 53 genesets, the enrichment level of top pathways (except for glutathione and drug metabolism associated with the glutathione S-transferase μ/GSTM family genes) increased as more genesets were included (Supplemental Information Fig. S2), indicating more complete coverage of these pathways exists. Furthermore, additional canonical pathways (e.g., purine and pyrimidine metabolism, Jak/STAT, ERBB, NOTCH, and B-cell receptor signaling) show enrichment as more genesets are included (Supplemental Information Fig. S2).

Discussion

Recent reports from the literature are beginning to uncover the complex nature of immune response following influenza vaccination and to develop predictive models of observed biological responses, focusing largely on antibody titers (HAI) as the currently accepted correlate of protection. [9–13] We and others have reported on the link between influenza virus-specific serological status and response (HAI and neutralizing antibody titers) and memory B cell response related to vaccination in young and older populations [1, 42, 43]. For example, we have demonstrated the positive correlation between the early dynamics/ change (Day 3 compared to baseline relative to vaccination) in influenza-specific memory B cell ELISPOT response and the increase (relative to baseline) in HAI and neutralizing

antibody titers following influenza vaccination [1]. Mounting scientific evidence suggests the important influence of different immune variables, particularly memory B cell status, on biological responses following infection and vaccination and the largely recall nature of the influenza A/H1N1-specific antibody response.[13–15] Memory B cells specific for viral conserved epitopes are an important source of influenza vaccine-induced plasmablasts that give rise to neutralizing antibodies [13–15]. The current study is the first systems-level attempt, using peripheral blood mononuclear cell transcriptional profiling and memory B cell response measures over time following vaccination, to develop models of vaccine-induced memory B cell response and delineate key pathways and biological functions inherent to this response in older individuals. In our study, assessment of baseline, early and late post-vaccination transcriptomic changes using linear models (gene-to-biology approach) revealed significant per-variable associations of multiple metabolism and immune response genes with the observed memory B cell response in our cohort of older individuals (Table 1).

Baseline, early and late gene expression changes were characterized by the consistent association of lipid, cholesterol, and other metabolism-related genes with the observed peak memory B cell ELISPOT response (Day 28-Day 0). These genes include mevalonate [diphospho] decarboxylase (*MDV*), phosphomevalonate kinase (*PMVK*), phosphatidylinositol 4-kinase (alpha component, *PI4KA*), dehydrogenase/reductase (*DHRS13*), phospholipid phosphatase 3 (*PPAP2B*), phosphatidylinositol-4-phosphate 5-kinase (*PIP5K1B*), glucose pyrophosphorylase (*UGP2*), and other genes involved in lipid/carbohydrate metabolism and cell signaling.

In addition, early (Day 3–Day 0) gene expression changes also demonstrated the involvement of known immune function-related genes in the development of memory B cell response. Important genes include a serine/threonine kinase (*STK17B*), which is involved in regulation of T cell activation and in antigen receptor signaling; a member of the NF-kappa-B inhibitor family (*NFKBIA*), which is involved in inflammatory response and apoptosis; a pellino ubiquitin protein ligase (*PELI1*), which is involved in B cell activation and TLR/IL1R signaling events in innate immune response/ inflammation; and a suppressor of cytokine signaling (*SOCS*), which is a major regulator of inflammation and infection-related immune response. Interestingly, a related molecular signature consisting of five significant genes (*PELI1*, *NFKBIA*, *SOCS3*, *GMEB1* and *NCRNA00152/LINC00152)* has been previously associated with dendritic cells' (DCs) transcriptional response to TLR4 stimulation (LPS) (GSE2706)[44], which supports early engagement of pathogen-associated molecular patterns/PAMP receptors (e.g., TLRs) and NF-kB signaling events in APCs to promote antigen presentation, costimulation and T helper and/or other cellular function.

In addition to metabolism-related genes, late (Day 28 – Day 0) gene expression changes were also characterized by the association of important immune and adhesion/motility genes with memory B cell response after vaccination. Interesting genes demonstrating significant associations include *ITGB1BP1*, which is an integrin beta 1 binding protein involved in integrin-dependent cell adhesion and signaling; *PVRL3*, which is a poliovirus receptor acting as an adhesion molecule at adherens junctions with a role in motility and cell proliferation; *NDFIP2*, which is a Nedd4 family interacting protein involved in ubiquitin-dependent protein sorting/trafficking, protein ubiquitation and regulation of I-kappaB

kinase/NF-kappaB signaling; and *CNPY4*, which is a chaperone involved in cell surface expression of TLRs.

Late transcriptional dynamics (associated with memory B cell response development) also revealed a molecular signature (*TCF4*; *PMVK*; *UGP2*, *SEPW1*, and *TMED7*) consistent with gene expression changes previously observed in DCs (30 min vs. 12 h) after TLR4 stimulation (GSE17721) [45], demonstrating the importance of metabolic genes (and genes with no known immune function) in PAMP receptors-associated immune responses/ processes.

Our complementary knowledge-driven (biology-to-gene) analytical approach also points to related biological functions and pathways attributed to transcriptional patterns, which are associated with memory B cell response (Table 2, Fig. 2), and involve early and late metabolic, cell migration/adhesion, MAP kinase and NF-kB cell signaling (chemokine/ cytokine signaling) and transcriptional regulation events. Related signatures (e.g., chemokine/cytokine signaling, pattern recognition receptor-associated signaling signatures, metabolic signatures) have been suggested as being important for mounting efficient antibody response (after influenza vaccination) by other studies. [9–13]

An interesting observation in our study is the identified early gene expression lipid signature (Table 2, Fig 2B) containing the *PDSS2* gene (decaprenyl diphosphate synthase subunit 2, which is involved in the biosynthesis of coenzyme Q/CoQ10); this was previously reported by Furman *et al.* to be associated with variations in antibody responses to influenza vaccine [46].

In our study, of particular interest is the involvement of genes related to cholesterol/sterol biosynthesis and membrane function in the regulation and mounting of memory B cells response after influenza vaccination. CoQ10 shares a biosynthetic pathway with cholesterol; functions as an electron transporter in the mitochondrial respiratory chain; and is important for energy production, beta-oxidation of fatty acids, biosynthesis of pyrimidines and other cellular processes. Recent studies have demonstrated the role of lipid metabolites, cholesterol biosynthesis and the host mevalonate pathway for respiratory syncytial virus infection, HIV uptake and replication, and also for the activation of human γδTCR cells during bacterial infection [47–49]. Even more exciting are the findings of a lipidomics profiling study of influenza infection in a mouse model (recapitulated in human samples in the same study), which demonstrated the association of specific lipid metabolites (5-lipoxygenase and 12/15-lipoxygenase) with the pathogenic and recovery stage of influenza infection, respectively, and with inflammatory response. This is solid evidence for the important role of lipid metabolite-related mechanisms and cellular functions for the course and/or severity of influenza [50]. Furthermore, lipid and, in particular, cholesterol metabolism and homeostasis has been reported to affect lipid rafts, B cell development, maturation and function/signaling, T cell polarization and the function of dendritic cells [51]. Alterations in cholesterol biosynthesis and cholesterol metabolism are frequently observed during normal aging. This results in higher total and low-density lipoprotein/LDL cholesterol plasma levels, which are likely due to the increase of adipose tissue during aging, increased absorption of cholesterol with decreased breakdown (to bile acids) and clearance,

specific gene expression changes, and changes in hormonal levels. While the link between these age-related changes and the observed altered immunity to vaccines in older individuals is likely, further investigation is warranted in order to more fully understand its impact on our findings.

To summarize our results into biologically valuable knowledge, we employed complementary approaches to annotate the functional enrichment within genes showing statistical associations with the observed peak B-cell ELISPOT response after influenza vaccination. Term enrichment indicates common functions between prioritized genesets. Specifically, among the nine genesets/pathways with the strongest statistical association with B-cell ELISPOT response (listed in Table 2), three genesets/pathways (Biocarta SARS pathway, KEGG Maturity onset diabetes of the young, and Epithelial to mesenchymal transition) are centered around metabolism; two genesets/pathways (Hydrolase activity, acting on carbon-nitrogen bonds and Transferase activity, transferring alkyl or aryl groups) are comprised of three known protein complexes that perform modifications of epigenetic marks; and four genesets/pathways (Leukocyte migration, Cytokine and chemokine signaling, Reactome p75NTR recruits signaling complexes, MAPK activity) are centered around cytokine and chemokine MAP kinase and NF-kB signaling and the inflammatory response.

Pathway enrichment tests also demonstrated significant representation of genes from multiple signaling pathways, including pattern recognition receptor-induced signaling cascades (Toll-like receptor, NOD-like receptor, RIG-I-like receptor, T-cell Receptor, neurotrophin, and MAPK), due to a common group of genes shared by these pathways (*IKBKB, MAPK1, MAPK7, MAPK9, MAPK11, MAPK12, MAPK14, TGFB1, TGFB2, TGFB3, TRAF6, IL8, IL10, IRAK1, RIPK2*, and *NGFR*). In addition to the interleukins, other cytokine/chemokine genes (i.e., TGFβ family genes and *CCR2*) represent interesting genes with possible involvement d in the development of B cell response. The development, survival and activation of B cells is mediated through cooperative signals through the B cell receptor, innate receptors (TLR9 and other TLRs) and receptors for B-cell activating factor BAFF-R to activate NFkB, MAPKs and other signaling cascades. These signaling events (including the TGF beta activated *MAP3K7*, an important regulator of cooperative B cell stimulation by BCR and TLR9) then trigger B cell differentiation, activation and wide array of biological functions (proliferation, cytokine secretion etc.) [52, 53]. Thus, the identified genesets, pathways, and genes in our study can be directly related to the development and activation of B cell response. The canonical pathways indicated by the most significantly associated genesets persist across threshold levels, adding confidence to the identified biological functions (Supplemental Fig. S2).

As genes were not selected independently from one another (we began with genesets, biology-to-gene approach), the p-values from enrichment tests should be interpreted conservatively. The purpose of enrichment analysis, in this context, is to act as a descriptive framework and guide interpretation in order to better understand the possible underlying mechanisms and functional roles of statistically prioritized genes, genesets, and pathways.

An important limitation of our study is the possibility of false-positive associations/ predictors of memory B cell response. To abate this concern, we report both p-values and q-values (for the per-variable analysis), which allows readers to evaluate the level of evidence. We also use a knowledge-driven biology-to-gene approach as part of our analytical strategy to control for FDR (see Supplemental Information). Another important limitation of the study is, due to limited sample availability, our mRNA-Seq analysis was done in PBMCs rather than in specific cell subsets of interest (e.g., antigen-specific B cells). Information on gene expression in antigen-specific B and T cells (and in other cell subsets) would greatly enhance the in-depth understanding of the biological processes and mechanisms underlying influenza vaccine-induced immunity.

While the identification of a predictive (of protection against influenza) immune response signature/signatures is the ultimate goal of systems vaccinology, our study did not include predictive modeling of the immune outcome per se. However, ours is still the first study to assess and report transcriptional signatures associated with the development of memory B cell response after influenza vaccination; thus, our study provides the knowledge base and lays the foundation for future work in this direction. Follow-up replication (with a larger sample size) and functional studies (e.g., gene knockout animal studies highlighting the role of specific genes in B cell memory development/activation during vaccination, or the level of pathway activity) are warranted to validate our findings and to identify predictive signatures of influenza-specific memory B cell immune response and the underlying mechanisms that mediate the development of B cell immune memory.

In conclusion, to the best of our knowledge, this is the first systems biology study utilizing unbiased high-throughput transcriptional profiling and modeling approaches to identify signatures of memory B cell response following influenza vaccination, adding to the emerging knowledge on humoral immunity and immune memory to influenza vaccination. The knowledge gained may inform the design of novel or improved vaccines against influenza in older individuals by devising solutions to overcome genetic, immunologic and age-related restrictions to protective immunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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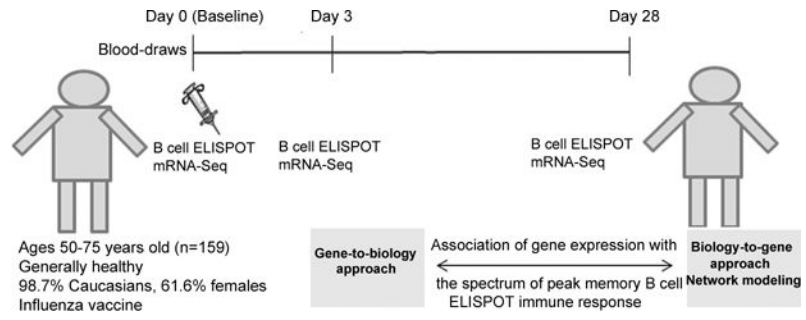
Fig. 1.
Study design
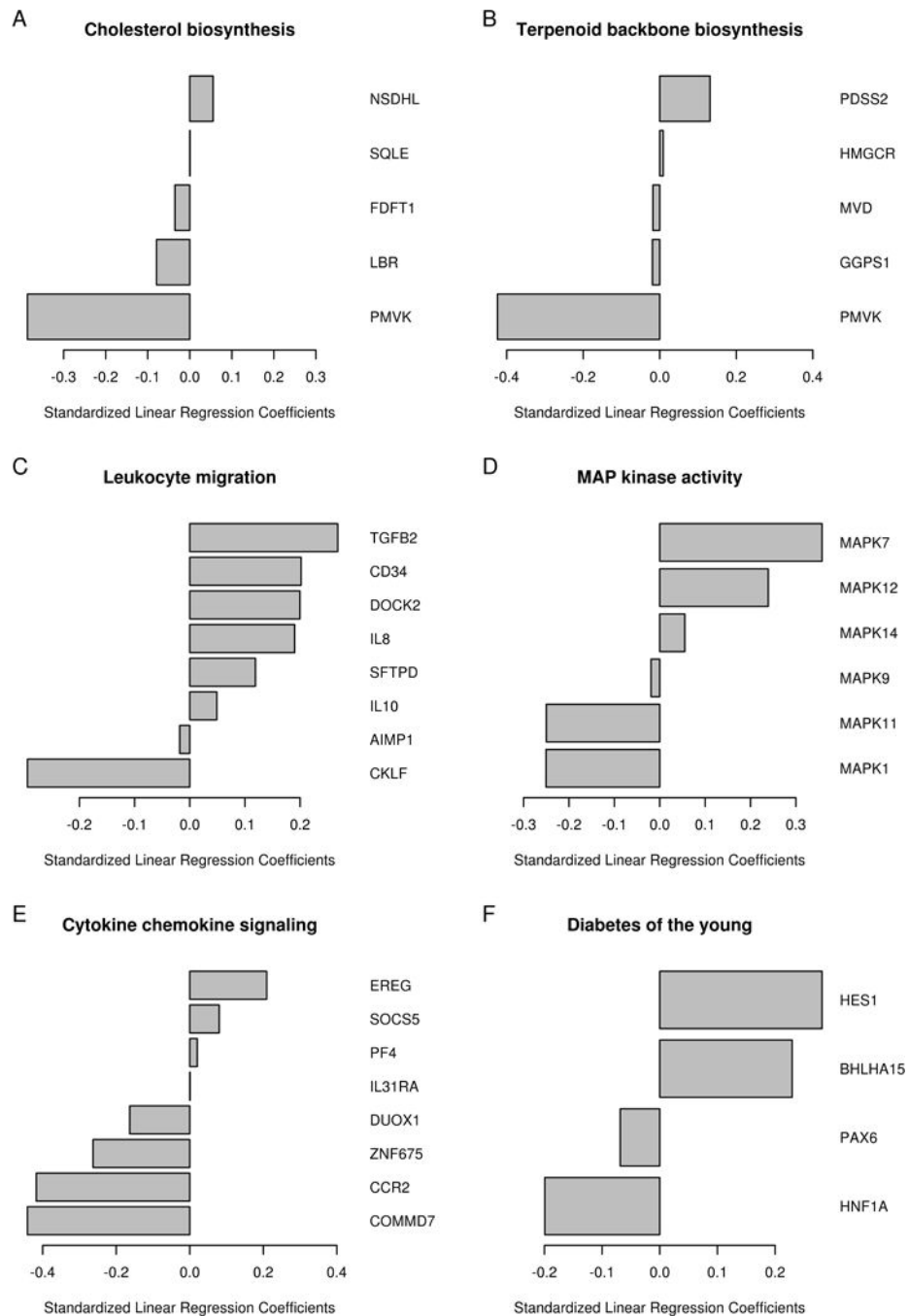
Fig. 2. Biological pathway-related genes, associated with peak memory B cell response after influenza vaccination

Penalized regression results of externally defined genesets at early and late gene expression changes associated with peak B cell ELISPOT response (Day 28-Day 0). A Cholesterol biosynthesis (Day 3-Day 0); B Terpenoid backbone biosynthesis (Day 3-Day 0); C Leukocyte migration (Day 28-Day 0); D MAP kinase activity (Day 28-Day 0); E Cytokine and chemokine signaling (Day 28-Day 0); F Diabetes of the young (Day 28-Day 0).
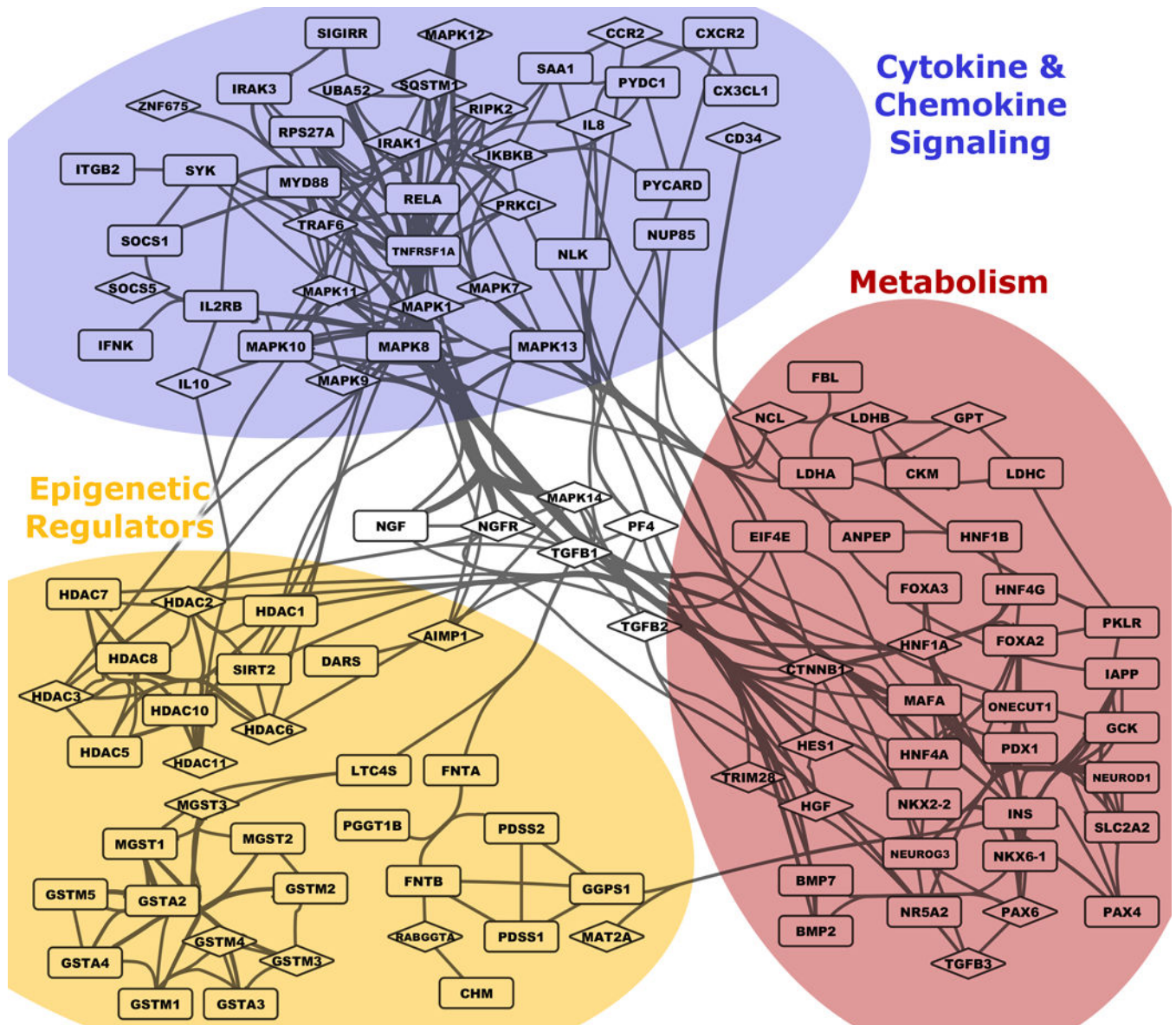
Fig. 3. Network modeling of biological pathway-related genes, predictive of peak memory B-cell ELISPOT response after influenza vaccination

Genes within the 9 genesets with strongest statistical association (with memory B-cell ELISPOT response) partition into three interrelated functional groups. A modified force-directed layout (AllegroLayout) was used to position genes, and then edges bundled to increase legibility. Genes included in GLMNET modeling are shown using diamond-shaped symbols, while other genes from the same genesets are shown as rectangles. Visualization performed in Cytoscape.

Table 1

Association of baseline, early and late gene expression changes with peak memory B cell ELISPOT response (Day 28 – Day 0) in older individuals

Gene Symbol	Coefficient	Std.Error	p-value	q-value	Gene name
Baseline gene expression (Day 0)					
MVD	2.11	0.46	7.585E-06	0.027	mevalonate (diphospho) decarboxylase
PLEKHM2	−4.40	0.98	1.363E-05	0.027	pleckstrin homology domain containing, family M (with RUN domain) member 2
FAM134C	2.12	0.48	1.509E-05	0.027	family with sequence similarity 134, member C
PJA2	−2.77	0.64	2.781E-05	0.027	praja ring finger 2, E3 ubiquitin protein ligase
NPEPL1	−0.92	0.21	2.987E-05	0.027	aminopeptidase-like 1
ATAD3C	0.63	0.15	3.48E-05	0.027	ATPase family, AAA domain containing 3C
PMVK	2.36	0.55	3.597E-05	0.027	phosphomevalonate kinase
MLXIP	−1.73	0.41	4.32E-05	0.027	MLX interacting protein
NCRNA00152	1.06	0.25	4.587E-05	0.027	long intergenic non-protein coding RNA 152
MTX1	−2.06	0.50	5.114E-05	0.027	metaxin 1
KIAA1958	−0.81	0.19	5.392E-05	0.027	KIAA1958
TMED7	−1.51	0.37	6.196E-05	0.027	transmembrane p24 trafficking protein 7
PIGU	2.10	0.51	6.361E-05	0.027	phosphatidylinositol glycan anchor biosynthesis, class U
CHML	−1.69	0.41	6.995E-05	0.027	choroideremia-like (Rab escort protein 2)
MAF	0.85	0.21	7.302E-05	0.027	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog
CCDC6	−2.34	0.58	7.728E-05	0.027	coiled-coil domain containing 6
PHF19	2.22	0.55	7.764E-05	0.027	PHD finger protein 19
PI4KA	−3.31	0.82	7.902E-05	0.027	phosphatidylinositol 4-kinase, catalytic, alpha
RDH16	0.90	0.22	8.381E-05	0.027	retinol dehydrogenase 16 (all-trans)
ZFAT	−1.56	0.39	9.193E-05	0.027	zinc finger and AT hook domain containing
IL32	0.74	0.18	9.288E-05	0.027	interleukin 32
NFATC3	1.65	0.41	0.00010	0.027	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
CERS6	−1.24	0.31	0.00010	0.027	ceramide synthase 6
SNRPC	2.04	0.51	0.00010	0.027	small nuclear ribonucleoprotein polypeptide C
LOC400927	−1.39	0.35	0.00011	0.027	TPTE and PTEN homologous inositol lipid phosphatase pseudogene
LRFN2	0.42	0.11	0.00011	0.027	leucine rich repeat and fibronectin type III domain containing 2

Gene Symbol	Coefficient	Std.Error	p-value	q-value	Gene name
HDHD3	1.03	0.26	0.00011	0.027	haloacid dehalogenase-like hydrolase domain containing 3
SERPINF1	−1.03	0.26	0.00011	0.027	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1
CLPB	−1.30	0.33	0.00012	0.027	ClpB homolog, mitochondrial AAA ATPase chaperonin
C9orf100	1.32	0.33	0.00012	0.027	Rho guanine nucleotide exchange factor (GEF) 39
Early gene expression (Day 3 – Day 0)					
PMVK	−2.80	0.61	7.578E-06	0.039	phosphomevalonate kinase
ZNF780B	−1.21	0.27	1.606E-05	0.039	zinc finger protein 780B
BLCAP	−2.73	0.62	2.251E-05	0.039	bladder cancer associated protein
TESK2	2.25	0.52	2.877E-05	0.039	testis-specific kinase 2
NFKBIA	1.21	0.29	4.615E-05	0.039	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
STK17B	2.04	0.49	5.329E-05	0.039	serine/threonine kinase
TMEM99	−0.90	0.22	5.591E-05	0.039	transmembrane protein 99
FCHSD2	2.07	0.50	5.69E-05	0.039	FCH and double SH3 domains 2
H3F3C	1.61	0.39	6.046E-05	0.039	H3 histone, family 3C
LRP11	−1.36	0.34	7.822E-05	0.039	low density lipoprotein receptor-related protein 11
DHRS13	−1.84	0.45	7.981E-05	0.039	dehydrogenase/reductase (SDR family) member 13
MAP4	−2.52	0.63	9.193E-05	0.039	microtubule-associated protein 4
PLEKHM2	3.90	0.97	9.541E-05	0.039	pleckstrin homology domain containing, family M (with RUN domain) member 2
CCDC6	2.24	0.56	9.984E-05	0.039	coiled-coil domain containing 6
USP6NL	1.33	0.33	0.0001096	0.039	USP6 N-terminal like
ZNF233	−0.74	0.19	0.0001107	0.039	zinc finger protein 233
MAP1LC3B2	1.27	0.32	0.0001153	0.039	microtubule-associated protein 1 light chain 3 beta 2
GMEB1	2.07	0.53	0.0001267	0.039	glucocorticoid modulatory element binding protein 1
C20orf134	−0.93	0.24	0.0001305	0.039	actin-like 10
TTLL1	−1.70	0.43	0.0001309	0.039	tubulin tyrosine ligase-like family member 1
ANO8	1.58	0.40	0.0001349	0.039	anoctamin 8
EEF1E1-MUTED	−1.88	0.48	0.0001406	0.039	eukaryotic translation elongation factor 1 epsilon 1, muted homolog
PELI1	0.91	0.23	0.0001446	0.039	pellino E3 ubiquitin protein ligase 1

Gene Symbol	Coefficient	Std.Error	p-value	q-value	Gene name
FAM21C	1.06	0.27	0.0001458	0.039	family with sequence similarity 21, member C
RAD51C	−1.73	0.45	0.0001527	0.039	RAD51 paralog C
THAP3	−1.37	0.35	0.0001603	0.039	THAP domain containing, apoptosis associated protein 3
SOCS3	1.05	0.27	0.0001737	0.039	suppressor of cytokine signaling 3
CHMP4A	−1.74	0.45	0.000177	0.039	charged multivesicular body protein 4A
CDK4	−2.21	0.57	0.000177	0.039	cyclin-dependent kinase 4
ELF1	1.75	0.46	0.0001815	0.039	E74-like factor 1 (ets domain transcription factor)
Late gene expression (Day 28 – Day 0)					
CMBL	−1.30	0.23	7.919E-08	0.00019	carboxymethylenebutenolidase homolog (Pseudomonas)
DHRS13	−2.41	0.43	8.749E-08	0.00019	dehydrogenase/reductase (SDR family) member 13
SEPW1	−3.20	0.58	1.13E-07	0.00019	selenoprotein W, 1
KRT1	−0.47	0.09	2.422E-07	0.00024	keratin 1, type II
UGP2	−3.40	0.63	2.728E-07	0.00024	UDP-glucose pyrophosphorylase 2
GOLGA7B	−1.41	0.26	3.083E-07	0.00024	golgin A7 family, member B
SERGEF	−2.65	0.50	3.498E-07	0.00024	secretion regulating guanine nucleotide exchange factor
ITGB1BP1	−2.48	0.47	3.909E-07	0.00024	integrin beta 1 binding protein 1
TTLL1	−2.32	0.44	5.787E-07	0.00029	tubulin tyrosine ligase-like family member 1
LOC147804	−1.99	0.38	6.934E-07	0.00029	TPM3P9, tropomyosin 3 pseudogene 9
NDFIP2	−1.60	0.31	7.938E-07	0.00029	Nedd4 family interacting protein 2
PPAP2B	0.86	0.17	7.991E-07	0.00029	PLPP3, phospholipid phosphatase 3
TRIM2	−1.15	0.22	8.582E-07	0.00029	tripartite motif containing 2
MSX2P1	−1.18	0.23	9.238E-07	0.00029	msh homeobox 2 pseudogene 1
CHMP4A	−2.22	0.44	9.387E-07	0.00029	charged multivesicular body protein 4A
FAM195B	−3.29	0.65	1.002E-06	0.00029	family with sequence similarity 195, member B
STX8	−3.29	0.65	1.032E-06	0.00029	syntaxin 8
TCF4	1.29	0.25	1.066E-06	0.00029	transcription factor 4
C14orf1	−2.15	0.42	1.199E-06	0.00031	chromosome 14 open reading frame 1
LOC388692	−1.41	0.28	1.362E-06	0.00032	uncharacterized LOC388692
KIAA0408	−1.11	0.22	1.373E-06	0.00032	KIAA0408

Gene Symbol	Coefficient	Std.Error	p-value	q-value	Gene name
CFH	−1.10	0.22	1.404E-06	0.00032	complement factor H
PCDHGC3	0.86	0.17	1.512E-06	0.00032	protocadherin gamma subfamily C, 3
CNPY4	−2.04	0.41	1.568E-06	0.00032	canopy FGF signaling regulator
LOC283174	−0.97	0.20	1.665E-06	0.00032	uncharacterized LOC283174
ZNF32	−2.15	0.43	1.695E-06	0.00032	zinc finger protein 32
PECR	−2.52	0.51	1.851E-06	0.00032	peroxisomal trans-2-enoyl-CoA reductase
PVRL3	−1.20	0.24	1.911E-06	0.00032	poliovirus receptor-related 3
BLCAP	−2.86	0.58	1.923E-06	0.00032	bladder cancer associated protein
PIP5K1B	1.22	0.25	1.958E-06	0.00032	phosphatidylinositol-4-phosphate 5-kinase, type I, beta

Linear model results of the top 30 genes associated with peak B cell ELISPOT response (Day 28 – Day 0). Baseline models include baseline gene expression as independent variables; early models include change in gene expression from baseline to Day 3 as independent variables; and late models include change in gene expression from baseline to Day 28 as independent variables. The coefficient is the change in peak B cell ELISPOT response per one unit change in the independent variable. The p-value is the statistical significance from the regression model, while the q-value is the false discovery rate as described in the Statistical methods.

Table 2

Pathways and biological functions associated with memory B cell response after influenza vaccination

Geneset/Pathway name	MSE[a]	r[2b]	Genes
Early (Day 3 – Day 0) gene expression changes, predictive of peak memory B cell ELISPOT response			
Kegg Terpenoid backbone biosynthesis	2.007	0.085	*PMVK;GGPS1;MVD;HMGCR;PDSS2*
Basement membrane	2.028	0.075	*FBN1;SMC3;LAMC1;EFEMP2;SNTB1*
Reactome Cholesterol biosynthesis	2.036	0.072	*PMVK;LBR;FDFT1;SQLE;NSDHL*
Late (Day 28 – Day 0) gene expression changes, predictive of peak memory B cell ELISPOT response			
Leukocyte migration	1.980	0.097	*CKLF;AIMP1;IL10;SFTPD;IL8;DOCK2; CD34;TGFB2*
Kegg Maturity onset diabetes of the young	1.990	0.093	*HNF1A;PAX6;BHLHA15;HES1*
Transferase activity, transferring alkyl or aryl (other than methyl) groups	1.990	0.093	*MGST3;GSTM3;GSTZ1;GSTM4;RABGGTA;MAT2A*
Biocarta SARS pathway	1.997	0.090	*LDHB;GPT;NCL*
MAP kinase activity	1.999	0.089	*MAPK1;MAPK11;MAPK9;MAPK14;MAPK12;MAPK7*
Reactome p75NTR recruits signaling complexes	2.005	0.086	*PRKCI;UBA52;TRAF6;NGFR;SQSTM1;IRAK1;RIPK2;IKBKB*
Cytokine and chemokine mediated signaling pathway	2.007	0.085	*COMMD7;CCR2;ZNF675;DUOX1;IL31RA;PF4;SOCS5;EREG*
Epithelial to mesenchymal transition	2.013	0.082	*S100A4;TRIM28;TGFB3;HGF;TGFB1;CTNNB1;HNRNPAB;TGFB2*
Hydrolase activity, acting on carbon-nitrogen (not peptide) bonds, in linear amides	2.016	0.081	*GLS2;HDAC3;HDAC11;HDAC2;HDAC6*
Regulation of protein polymerization	2.029	0.075	*MAPT;TMSB4Y;MAPRE1*

[a] MSE mean squared error,

[b] Estimated r^2

Penalized regression results of externally defined genesets at early and late gene expression changes associated with peak B cell ELISPOT response (Day 28-Day 0). The independent variables are the change in gene expression from baseline to Day 3 (early) and baseline to Day 28 (late) for each geneset as described in Statistical methods.