Comparison of immunological characteristics of peripheral, splenic and tonsilar naïve B cells by differential gene expression meta-analyses

Kaj Chokeshai-u-saha,[1] Cyrille Lepoivre,[2] Luca Grieco,[2] Catherine NGUYEN,[2,3] and Kiat Ruxrungtham[1,4]

Summary

Background: **Naïve B cells isolated from peripheral blood, spleen and tonsil are commonly used in human B cell studies. However, little has been written about their possible variations in immunological properties. This study compared differential gene expression in human naïve B subsets by meta-analysis using expression data available in Gene Expression Onimbus (GEO).**

Methods: **Gene expression files of the Affymetrix Human Genome U133A Array (Affymetrix) were downloaded to collect 21 total array data samples of peripheral naïve B cells (n=10), splenic naïve B cells (n=2), tonsilar naïve B cells (n=3), peripheral memory B cells (n=4) and splenic memory B cells (n=2). Prior to differential gene expression analyses, data were normalized in order to reduce non-biological variation among the datasets.**

Results: **Comparisons of peripheral naïve B cells with their splenic and tonsilar counterparts showed remarkable differences in terms of gene expression (29 and 202 genes, respectively). However, only minor differences were detected between splenic and tonsilar naïve B cells (10 genes), consistent with the clustering results classifying both of them as lymphoid naïve B cells. Differential gene expression results also implied higher stimulating states of lymphoid naïve B cells when compared with peripheral blood naïve B cells. These included enhanced expressions of CD27, CR2, EGR1, GADD45B, ICAM1, ICOSLG, IGHA, IL6, MMP9, SAMSN1, SMAD7, TNFAIP3, but reduced HLA-DOB expression.**

Conclusions: **Our findings suggest that results generated from peripheral naïve B cells may not always be applicable to the biological activities of other lymphoid naïve B cells. Nonetheless, further biological study is warranted.** ***(Asian Pac J Allergy Immunol 2012;30:326-30)***

Key words: *Naïve B cell subset, peripheral blood, splenic, tonsillar, meta-analysis, differential gene expression; memory B cell; immunoproperties*

From 1. Division of Allergy and Clinical immunology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
2. Inserm U1090, TAGC, Parc Scientifique de Luminy, Marseille, France
3. Aix Marseille University, Marseille, France
4. Vaccine and Cellular Immunology Laboratory, Vaccine Research Center (ChulaVRC), Chulalongkorn University, Bangkok, Thailand
Corresponding author: Kiat Ruxurngtham
E-mail: kiat.r@chula.ac.th
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Introduction

The common sample sources for the study of human naïve B cells are peripheral blood, spleen and tonsil. Although naïve B cells isolated from these sources are known to share several common characteristics,[1,2] differences among them are a possibility. Mature naïve B cells normally recirculate from the spleen throughout the body's lymphoid organs where they may encounter antigens (Ag) and further differentiate into Ag specific memory B cells or plasma cells. Most cellular factors required for these mechanisms are thus locally provided only in lymphoid niches, but not peripheral blood.[3,4] This should affect lymphoid naïve B cells, and is likely to modulate their intrinsic properties which are absent in peripheral naïve B cells.

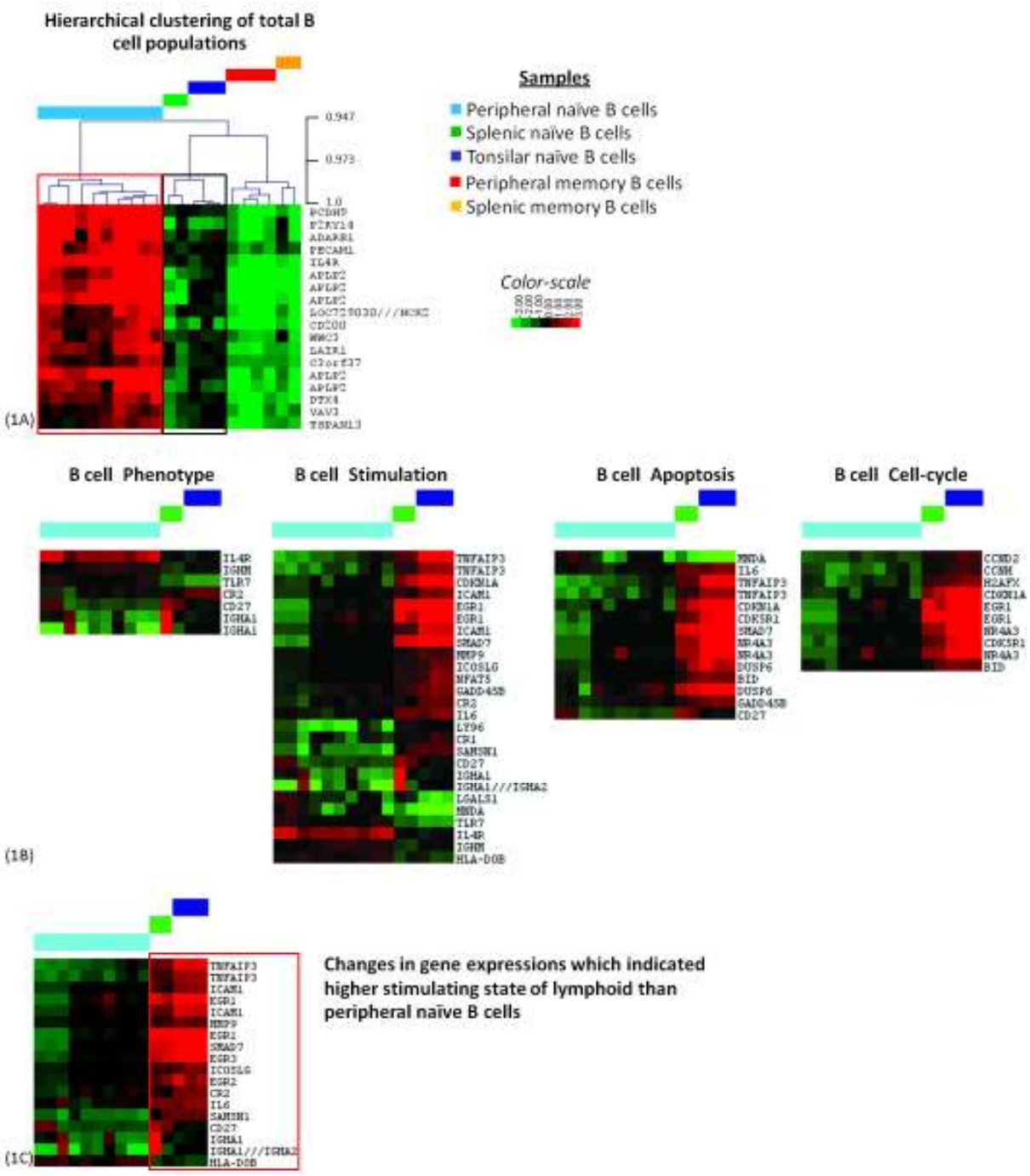

Figure 1. Expression profiles of the peripheral naïve B cell subset were different from those of the lymphoid naïve B cell subsets (splenic and tonsilar naive B cell subsets)
1A). Hierarchical clustering results based on differentially expressed genes (18 genes were shown here) within B cell populations. Separation between peripheral naïve B cells and other lymphoid naïve B cells (splenic and tonsilar naïve B cells) is presented. Peripheral and lymphoid naïve B cells were highlighted using red and black squares. 1B). Heatmaps of differentially expressed genes within the naïve B cell subsets. Differential gene expressions associated with common B cell phenotypes, B cell stimulation, B cell apoptosis and B cell-cycle were merged to visualize the naïve B cell subsets used in this study. 1C). Changes in gene expression which indicated the higher stimulation state of lymphoid as opposed to peripheral naïve B cells are further selected and shown. To draw the heatmaps, data were median centered on the rows.

Further characterization of different naïve B cell subsets is therefore essential. However, a direct comparative study has several limitations including ethical consideration (in relation to obtaining tissue samples) and inadequate sample sizes making it impossible to perform a variety of comparisons. It is thus important to use a sensible screening method to investigate variations in these characteristics. The availability of the microarray gene expression database, means that gene expression meta-analysis can be carried out to screen genes and underlying metabolic characteristics specific to particular cell types or perturbations. By applying this method, our study aimed to identify differential gene expression levels in human peripheral, splenic and tonsilar naïve B cells.

Materials and methods

B cell expression data

Three datasets are available in the Gene Expression Onimbus (GEO) from Abbas et al (GSE22886, n=11),[5] Longo et al. (GSE12845, n=6)[6] and Good et al. (GSE13411, n=4)[7] were selected and retrieved to put together 21 total array data samples. These arrays included Peripheral naïve B cells (n=10), Splenic naïve B cells (n=2), Tonsilar naïve B cells (n=3), Peripheral memory B cells (Per Bmem) (n=4) and Splenic memory B cells (Splenic Bmem) (n=2).

Affymetrix Gene Chip Comparison

Data were pre-treated for each dataset singularly, using the gcRMA method for background noise adjustment, followed by a quantile-based normalization performed at probe level. A Median Polish method-based procedure was then applied to obtain gene expression levels by summarizing probeset normalized values. Additionally, to reduce non-biological variation among microarray studies (batch effects), a normalizing algorithm, ComBat[8], was applied before performing comparative analysis for which the attenuated batch effects were proved by Principal Component Analysis (PCA). Hierarchical Clustering of samples was then performed based on the Pearson's centered correlation coefficient. MeV and Cluster 3.0 software and Java Treview software were used to visualize the results.

To select genes with significant differences of expression between cell types, we used a combination of two methods, the Kruskal-Wallis test and Local Pooled Error (LPE) test which are suitable for microarray data with low replicate numbers. The *p*-values obtained from both tests were corrected through the Benjamini-Hochberg formula. The Kruskal-Wallis test was applied to select the genes varying most significantly among the different cell types. The False Discovery Rate (FDR) threshold for this test was set to 0.05. The Local Pooled Error (LPE) statistical test was applied for identifying the most significant differentially expressed genes between couples of naïve B cell types. The FDR threshold for this test was set to 10^{-5}.

A gene was considered differentially expressed in a comparison if it was declared significant by both the algorithms used and, at the same time, its fold-change was equal to or greater than 2. Multiple experiment viewer (MeV) software and the R programming language were used for these analyses, with the help of existing packages for R ("gcrma", "LPEadj") available on the Bioconductor website (http://www.bioconductor.org). Acquired differentially expressed gene lists of interest were further categorized according to known B cells' characteristics.

Results

All B cell samples were clustered based on total differentially expressed probes (Kruskal-Walis, FDR <0.05) (Figure 1A). While splenic and tonsilar naïve B cells showed a strong correlation as lymphoid naïve B cells, exclusive clustering of peripheral naïve B cells from other naïve B cell subsets was demonstrated. Expression comparisons among naïve B cell subsets were subsequently performed. Three pairs of comparisons (LPE and BH correction, FDR $<10^{-5}$) were made: "splenic versus tonsilar naïve B cells", "peripheral versus splenic naïve B cells", "peripheral versus tonsilar naïve B cells". The numbers of detected differentially expressed probes from each comparison were 12, 31 and 235, corresponding to 10, 29 and 202 genes, respectively. Several differentially expressed genes were related to "B cell phenotypes", "B cell stimulation", "B cell apoptosis" and "B cell Cell-cycle" characteristics (Figure 1B). Of these differences, changes in gene expressions which indicated a higher stimulating state of lymphoid as opposed to peripheral naïve B cells were further selected and shown (Figure 1C). Since changes in several gene expressions are evident after B cell stimulation, we categorized differential gene expressions accordingly. Interestingly, several changes in gene expressions indicated that lymphoid naïve B cells acquired more stimuli than peripheral naïve B cells, especially those of Ag and CD40L which respectively implied antigen recognition and interaction with localized T cells (Table 1).

Table 1. Changes in gene expressions which indicated a higher stimulation state in lymphoid as opposed to peripheral naïve B cells

Genes	Encoded molecules	Function	Up/Downregulated upon stimulation	Stimuli
CD27	CD27	B cell costimulatory molecule associated with differentiation, proliferation and apoptosis	Upregulated	CD40L+antigen
CR2	Complement receptor 2	Immune complex recognition	Upregulated	antigen
EGR1	Early growth response protein 1	Cell-cycle progression, and downregulation of Fas and CD23 expression	Upregulated	antigen
GADD45B		Protect Fas-induced apoptosis	Upregulated	CD40L
HLA-DOB	Human Histocompatibility Leukocyte Antigen (HLA)-DOB	Inhibit peptide loading on HLA-DM	Downregulated	CD40L
ICAM1	Intercellular Adhesion molecule-1 or CD54	Intercellular adhesion/costimulation of B cell during Ag presentation to helper T cell (Th)	Upregulated	CD40L
ICOSLG	ICOS ligand	Help with follicular helper T cell (Tfh cell) differentiation	Upregulated	CD40L
IGHA	Immunoglobulin heavy constant alpha chain	Major component of IgA	Upregulated	antigen
IL6	Interleukin 6 (IL-6)	Important proinflammatory cytokine	Upregulated	antigen, Bacteria
MMP9	Matrix metallopeptidase 9	Degrade basement membrane and cellular matrix during B cell migration	Upregulated	TNFα
SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1	Negative regulator of B cell activation and differentiation	Upregulated	IL-4, CD40L, antigen
SMAD7	SMAD protein 7	Protect cell from TGFβ-induced growth inhibition and apoptosis	Upregulated	TGFβ, CD40L
TNFAIP3	Tumor necrosis factor alpha-induced protein 3	Negative regulator of NFkB involving with several B cell mechanisms	Upregulated	CD40L

Discussion

The gene expression profiles of the peripheral naïve B cell subset revealed a different pattern from those of splenic and tonsilar naïve B cell subsets which could be clustered together as lymphoid naïve B cells (Figure 1A). Interestingly, expression profiles of lymphoid naïve B cells implied previous stimulation when compared with peripheral naïve B cells (Figure 1C and Table 1).

The differences between peripheral and lymphoid naïve B cells supported the idea that lymphoid naïve B cells were more activated than peripheral blood B cells. A possible explanation is that naïve B cells located in lymphoid tissues might encounter stimuli (particularly those associated with Ag recognition and Ag presentation to T cells) more often than peripheral blood cells. In conclusion, our results suggest that there are several possible differences in biological characteristics between peripheral and lymphoid naïve B cell subsets that require further investigation at the protein and functional levels.

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