Distinct and mutually exclusive Ca ++ flux- and adenyl cyclase-inducing gene expression profiles of G-Protein-Coupled Receptors on human antigen-specific B cells

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February 1, 2023

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B cells play an essential role in allergies by producing allergen-specific IgE, which is a prerequisite for allergen-induced degranulation of mast cells (MCs) and basophils. MCs, basophils, dendritic cells and bacteria are capable of releasing inflammatory mediators including histamine. Histamine is a bioactive amine that exerts its function through binding to histamine receptors (HRs), which are 7-transmembrane G-protein-coupled receptors (GPCRs). There are four types of HRs (HR1-4), wherein HR1 ligation triggers Ca2+ mobilization, HR2 stimulates and increases cAMP concentrations, and HR3 and HR4 inhibit cAMP accumulation[1]. In the presence of histamine in the environment, high affinity HR1is triggered causing cellular activation, followed by expression of 10 times lower affinity HR2 to regulate the over-inflammatory events. These HRs trigger different intracellular events upon activation, with HR1 as a Ca2+ flux-inducing activating receptor and HR2 as an adenyl cyclase-stimulating suppressive receptor [1,2]. Therefore, to explore the response of B-cells in allergic diseases, we analyzed the expression profile of HRs and other GPCRs in B cell clones. We hypothesized that the expression profile of HRs (HR1+ vs HR2+ B cell clones) is associated with significant changes in the expression profile of other GPCRs that govern the downstream cascade of pathways associated with cAMP signaling or Ca2+ mobilization.

A total of 27 IgG1 and IgG4 expressing B cell clones were isolated for gene expression analysis under BCR stimulated and unstimulated conditions **(Figure 1A** and **Online Supplementary Methods)** . Interestingly, we observed B-cell clones with mutually exclusive expression profile of *HRH1* and *HRH2* genes (**Figure 1B),** with more *HRH1+* B-cell clones in BCR-stimulated samples than unstimulated samples. The

subsequent*HRH1+* vs *HRH2+* differential gene expression analysis (**Figure 1C**), reveal 27 differentially expressed (DE) GPCRs in unstimulated samples, with up-regulated *P2RY13* and *C5AR1*genes in *HRH2* + B-cell clones (**Figure 2A)** , which are associated with the cAMP signaling and suppressive pathway[3,4]. To further prioritize the DE GPCRs specifically associated with Ca2+ and cAMP signaling pathways, we reconstructed the co-expression networks and performed the weighted degree analysis across *HRH1+* vs *HRH2+* clones. The analysis reveals that the purinergic receptor family of GPCRs (i.e. *P2RY1* , *P2RY13*) and complement component 5a receptor family of genes (i.e. *C5AR1* and *C5AR2*) share highest degree of interactions. These genes are up-regulated in*HRH2+* samples and are well-known to affect cAMP signaling pathway[3,4] (**Figure S1A**). Intriguingly, we also observed upregulation of *GPR35* in *HRH2* + B cells, which is associated in maintaining a low baseline Ca2+ level[5]. Similarly, we also observed up-regulation of *GPR68* and *GPR171* in *HRH1* + B cells; both are known to stimulate Ca2+ flux (**Online Supplementary Discussion)** .

Similarly, 28 GPCRs were differentially expressed in BCR-stimulated samples (**Figure 2B**), including higher expression of serotonin receptor type 1A (*HTR1A*) and *HCAR1* (or *GPR81*) in*HRH2+* samples, with a cAMP-linked suppressive function. In addition, we also observed upregulation of complement component 5a receptor family of genes (i.e., *C5AR1* and *C5AR2*) and *GPR35* , in agreement with the trend observed in unstimulated*HRH2* + B-cell clones. Surprisingly, we observed a higher expression of prostaglandin E2 receptor subtype EP4 (*PTGER4)* and adenosine A2A receptor (*ADORA2A*) in *HRH2+* samples[3,6], which are known to be associated with activation of cAMP production and share the highest strength of interactions with the cAMP signaling sub-network (**Figure S1B**). Among the up-regulated genes in *HRH1* + samples, we found three Ca2+ mobilizing genes, i.e., *GPR34* ,*P2RY10* and *PTAFR* .

The results reported in this study provides data for a novel hypothesis suggesting investigation of co-expressed genes that may play important synergistic or antagonistic regulatory roles in B-cell function.

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Figure Legends

Figure 1 . Study design and differential gene expression of histamine receptors. **a.** The study design used for isolation and sorting of B-cells clones for gene expression analysis and the data analysis workplan for identification and prioritization of GPCRs.**b.** Mutually exclusive expression profile of *HRH1* and*HRH2* in both unstimulated and BCR-stimulated samples. The dotted box represents the threshold within which samples were considered as double negative, i.e., *HRH1- /HRH2-* . **c** . Volcano plots to highlight differentially expressed GPCR genes in *HRH1+*vs *HRH2+* samples in both unstimulated and BCR-stimulated samples. The red dots represent significantly differentially expressed genes ($p < 0.05$ & log-foldchange > 0.5).

Figure 2 . Differentially expressed GPCRs in *HRH1+* vs*HRH2+* samples. **a.** Heatmap showing expression profile of differentially expressed GPCR genes in all the BCR-stimulated samples, including double negative samples. The top two panels represent the*HRH1+* and *HRH2+* status of each sample. **b.** The boxplot showing topmost significantly differentially expressed GPCR genes in ($p < 0.001$) in BCR-stimulated samples. **c.**Heatmap showing expression profile of differentially expressed GPCR genes in all the unstimulated samples. The annotations are same as Figure2a. **d.** The boxplot showing topmost significantly differentially expressed GPCR genes in ($p < 0.001$) in unstimulated samples.

Conflict of Interest: Authors declare no conflict of interest.

Author Contribution: IC: Writing, review & editing, formal analysis; AK: Writing, review &editing, software and formal analysis; PS, LB, SRS: review & editing; MY: formal analysis; KN: supervision, review & editing; WvdV: review & editing, conceptualization, method, provide data; CAA and MA: review & editing, conceptualization, supervision

Figure1

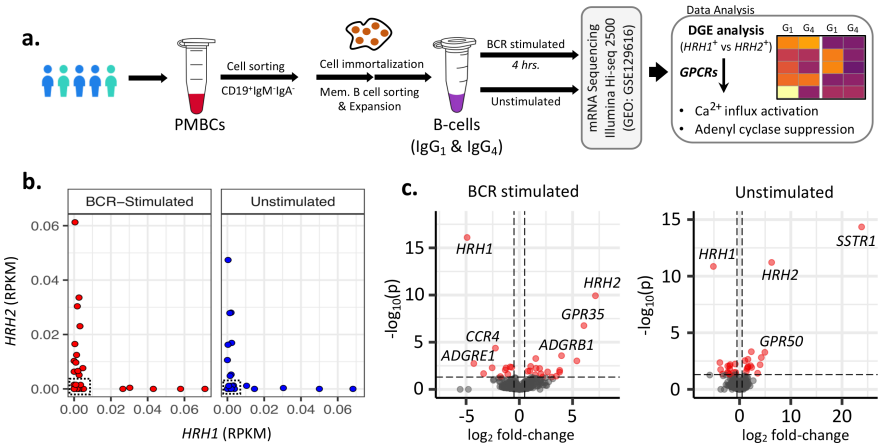

Figure2