Title: Enlightening human B-cell diversity

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Keywords: B cells; human; mass cytometry; single cell; multi-omic; cell atlas

Abbreviations: Ig, immunoglobulin; SHM, somatic hypermutation;

Since their discovery in the 60s´, B cells have been extensively studied because of their unique and critical role in immunoglobulin (Ig) production. In addition, B cells contribute to other aspects of the immune response via antigen presentation to T cells and cytokine production. Despite the intensive study of B cells, its classification in different subsets based on surface markers remained controversial.

Historically, B cells in the periphery (*i.e.*, blood, secondary lymphoid organs) were classified according to their maturation stage and the Ig isotype expressed in 5 populations: transitional, naive, non-switched memory, switched memory and plasma cells. Importantly, this classification does not reflect precisely functional aspects (*i.e.*, metabolism, cell signaling, tissue of origin), and in some cases the markers used to identify B-cell subsets were faulty. For example, CD27 was long considered a classical marker of memory B cells and associated with somatic hypermutation (SHM), however some memory B cells lack CD27 expression.[1] In this context, B-cell biologists were demanding to update the criteria used to classify B cells and the inclusion of novel functionality markers, which has been possible with the advent of single cell multi-omics.

Glass *et al.* developed a new strategy for human B-cell classification using mass cytometry. They analyzed the expression of 351 markers on B cells from 4 different tissues (tonsils, lymph nodes, peripheral blood and bone marrow) and defined 12 populations based on the 98 markers that were expressed by B cells (**Figure 1**).[2] This new classification defines the B-cell maturation stage and also informs of the functional and metabolic profile. After a massive analysis, they showed that 10 of these 12 populations were detected in peripheral blood (frequency ≥1%) and could be distinguished with a combination of 8 markers (CD45, CD19, CD38, CD73, CD45RB, CD27, CD11c and CD95).[2]

Among these 8 markers, CD45RB was shown to be useful for the identification of memory B cells. Indeed, its expression, in combination with that of CD27, correlated better with the degree of SHM than CD27 alone. The use of CD45RB for memory B cell identification might solve the discrepancies draining from the CD27-based classification.[2]

Furthermore, the authors updated the classification of memory B cells showing 6 different populations. Within them, the $CD19^{hi}$ $CD11c^{+}$ subset was previously reported as relevant in autoimmunity and infection.[3] Moreover, they established CD95 as a new marker for a B cell subset. The $CD95^{+}$ memory B cell subset showed a high response after stimulation, indicating that these cells are effector memory B cells.[2]

Of the 4 tissues studied, they found that only $CD39^{+}$ B cells were tissue specific. This population, exclusive of the tonsils, appeared to be memory B cell precursors due to the expression of memory-related markers such as CD11c. However, according to the literature, $CD39^{+}$ B cells were considered as regulatory B cells.[4] In addition, the authors did not find innate B1 cells, being a population widely characterized in humans.[5] In spite of the extensive marker analysis performed in this work and the new B-cell classification proposed, these discrepancies ensure further studies.

A central feature of this study is that it enables the cytometric identification of 10 B-cell subsets in peripheral blood with 8 surface markers. It opens the possibility to a large number of 8-10 color flow cytometry users, to work with this updated B-cell subset classification routinely. Consequently, one may expect to see in the near future the application of this updated B-cell classification for the analysis of B cells in different human pathologies including allergic diseases.

The identification of 6 subsets of memory B cells in humans by Glass *et al.*[2] may be particularly relevant in food allergy, where IgG^{+} memory B cells are thought to play a critical role in the maintenance of IgE immunity, both in mice and humans.[6] The analysis of allergen-specific IgG^{+} memory B cells with this new classification may advance our understanding of the IgE memory reservoir in food allergy. However, this will require the use of more sophisticated flow cytometry staining methods, beyond 8 colors, in order to incorporate allergen-specificity and *bona fide* Ig identification of memory B cells and plasma cells.[7,8]

Lastly, it has been recently reported a substantial presence of IgE-expressing cells in human mucosal sites including the stomach and duodenumin peanut allergic patients.[9] Clonally related IgE^{+} and non-IgE-expressing cell frequencies in tissues suggest local Ig switching, including transitions between IgA and IgE isotypes. The similarity of antibody sequences specific for the peanut allergen Ara h 2 was shared between patients, which indicates that common Ig genetic rearrangements may contribute to pathogenesis. These data support the notion that the gastrointestinal tract serves as a reservoir of IgE^{+} B lineage cells in food allergy. Therefore, it seems relevant to apply this approach, not only to peripheral blood, but to human gastrointestinal biopsies of food allergic patients even though it presents a considerable logistical challenge.[6]

Figure 1. Human B cells from blood, lymph nodes, tonsils and bone marrow of healthy donors were comprehensively analyzed by mass cytometry.[2] The expression of 351 surface molecules was assessed, 98 of which were expressed by human B cells. This analysis enabled the identification of 12 unique subsets of B cells, 10 of which were detected in peripheral blood (bottom left box) and 2 were exclusively detected in tonsils and lymphoid tissue (bottom right box). In addition, the isotype usage, VDJ sequence, metabolic profile, biosynthesis activity and cell signaling of the different B cell subsets was determined. VDJ, variable, diversity and joining gene segments.

Conflict of interest: The authors have no conflict of interest to declare.

Financial support: ISF is supported by a grant (RTI2018-101345-B-I00) from the MCIU/AEI/FEDER EU to YRC. RJS is funded by the Severo Ochoa Program (AEI/SEV-2017-0712).

Authorship: The authors approved the final version of the manuscript as submitted and agreed to be accountable for all aspects of the work.

Acknowledgements: The authors want to thank Dr. Anna Globinska for graphical abstract design.

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