ImmunoSpot® assays enable in-depth assessment of B cell reactivity against SARS-CoV-2 and seasonal influenza

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ABSTRACT

Background: Memory B cells constitute a critical component of humoral defense and are efficiently recruited into secondary immune responses upon antigen re-encounter. Expansion and subsequent differentiation of memory B cells into antibody-secreting cells (ASC) serves to rapidly increase antibody levels and limit dissemination of infectious agents. In contrast to the short half-life of secreted antibody and its eventual decline in the absence of continuous replenishment by ASC, memory B cells are long-lived and their precursory frequencies are stably maintained in the absence of antigen re-encounter. Consequently, measurement of antigen-specific memory B cell frequencies and characterization of their fine antigen specificity can provide unique insights into their recall potential and may additionally offer a more reliable and predictive correlate of protection.
Methods: Presently there are few techniques that enable facile identification of antigen-specific B cells at single-cell resolution and with high-throughput capacity. Unlike flow cytometric techniques, which depend upon surface B cell receptor-mediated acquisition of fluorescently-conjugated probes, the ImmunoSpot® approach permits identification of rare antigen-specific B cells based on the secretory foot-prints generated by individual ASC. Utilization of fluorescently-conjugated detection reagents in the ImmunoSpot® assay also facilitates multiplexing and parallel assessment of antigen-specific B cell Ig class or IgG subclass usage. Moreover, inverted ImmunoSpot® assays, in which soluble antigen is utilized as detection probe for revealing ASC-derived secretory foot-prints, enable assessment of functional affinity through titration of the antigen concentration. Additionally, incorporation of multiple antigen probes in the context of inverted ImmunoSpot® assays also permits evaluation of ASC cross-reactivity at single-cell resolution.
Results: In addition to evidencing underlying B cell memory against SARS-CoV-2 and seasonal influenza in subjects exhibiting low levels of circulating antibody reactivity, ImmunoSpot® assays are also economical in cell utilization and enabled parallel assessment of multiple antigens and Ig class usage using minimal precious cell material. Moreover, inverted ImmunoSpot® assays recapitulated both ASC reactivity and their relative precursor frequency, and facilitated assessment of functional affinity through titration of soluble antigen probes representing SARS-CoV-2 or influenza.
Conclusion: Our data illustrate the utility of ImmunoSpot® assays for detailed assessment of memory B cell reactivity and the requirement for cellular immune monitoring efforts to reveal what serum antibody may not.

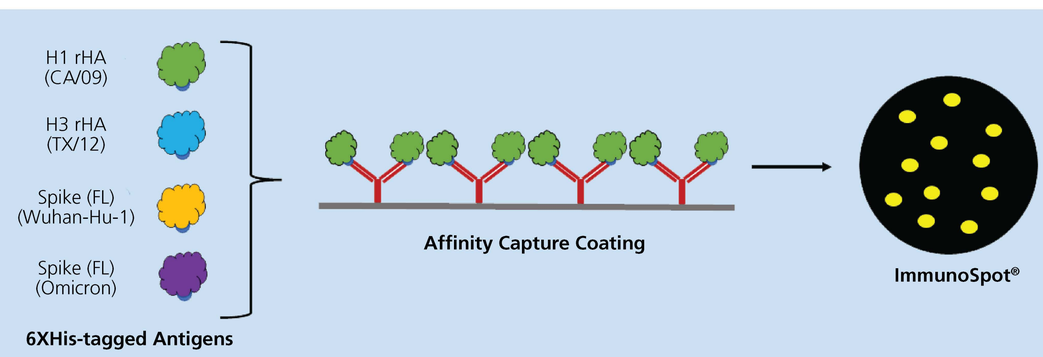

Fig. 1: Schematic representation of affinity capture-mediated antigen coating of ELISPOT/FluoroSpot membranes. The approach shown is suited for recombinant antigens that are hexahistidine (6XHis) tagged, such as influenza rHA or SARS-CoV-2 Spike proteins. The PVDF membrane on the bottom of the assay plate is first coated with an anti-His antibody, that in a subsequent coating step binds the His-tag of the added antigen. As the next step of the ELISPOT/FluoroSpot assay (not shown) the PBMC containing antibody secreting cells (ASC) are added. During the subsequent cell culture period, while all ASC (irrespective of their antigen specificity) secrete antibody, the immunoglobulin they release will be captured around antigen-specific B cells only. The plate bound secretory antibody footprint of each antigen-specific B cell is then detected in the final steps of the assay, and the spot forming units (SFU) are counted.

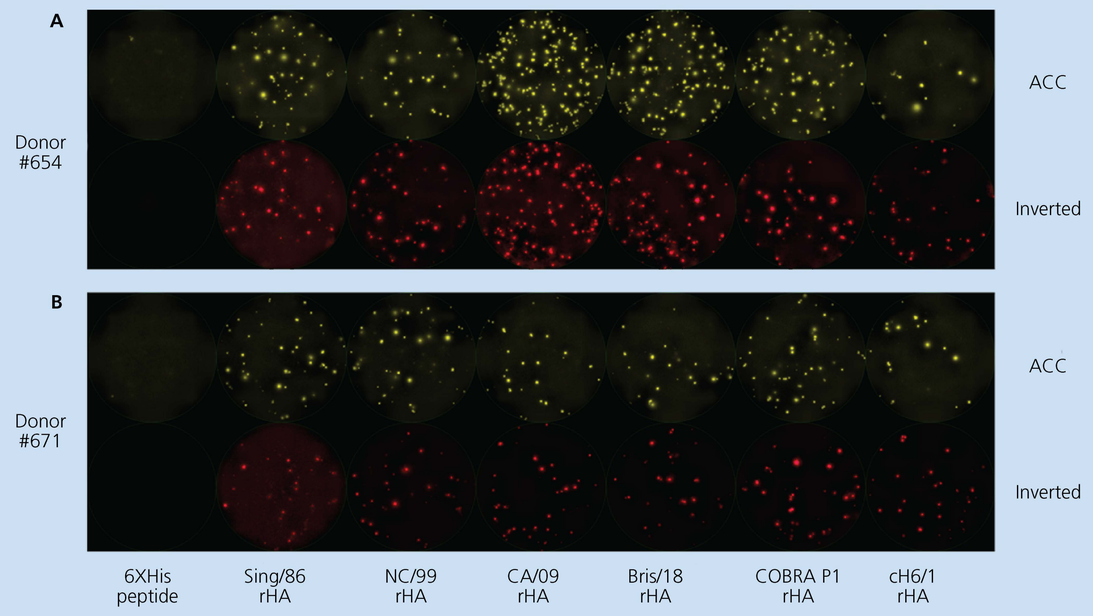

Fig. 4: Evaluation of B_{mem}-derived ASC reactivity against a panel of recombinant HA (rHA) proteins. (A and B) Representative well images depicting B_{mem}-derived IgG^+ ASC activity of two donors (#654 and #671) PBMC following pre-stimulation against rHA proteins (~1 x 10^5 cell input) representing past seasonal H1N1 influenza vaccine strains or universal influenza vaccine candidates (COBRA P1 or cH6/1). B_{mem}-derived IgG^+ ASC activity of Donor #654 and Donor #671 were evaluated against rHA proteins coated either through affinity capture (top row) or using an inverted assay approach (bottom row) in which rHA-6XHis probes were revealed using an anti-His detection system. The negative controls were either 6XHis peptide coated through affinity capture or 6XHis peptide used as the detection probe in the inverted assay. Abbreviations of H1N1 viruses are follows; Sing/86 = A/Singapore/6/1986, NC/99 = A/New Caledonia/20/1999, CA/09 = A/California/04/2009, Bris/18 = A/Brisbane/02/2018. COBRA P1 rHA represents a universal vaccine candidate that was generously provided by the laboratory of Ted M. Ross and has been described previously (PMID 26912624 and PMID 31811019). The cH6/1 rHA represents a stem-based immunogen and was previously described (PMID 31022693). Contrast enhancements of well images were performed to aid their visualization.

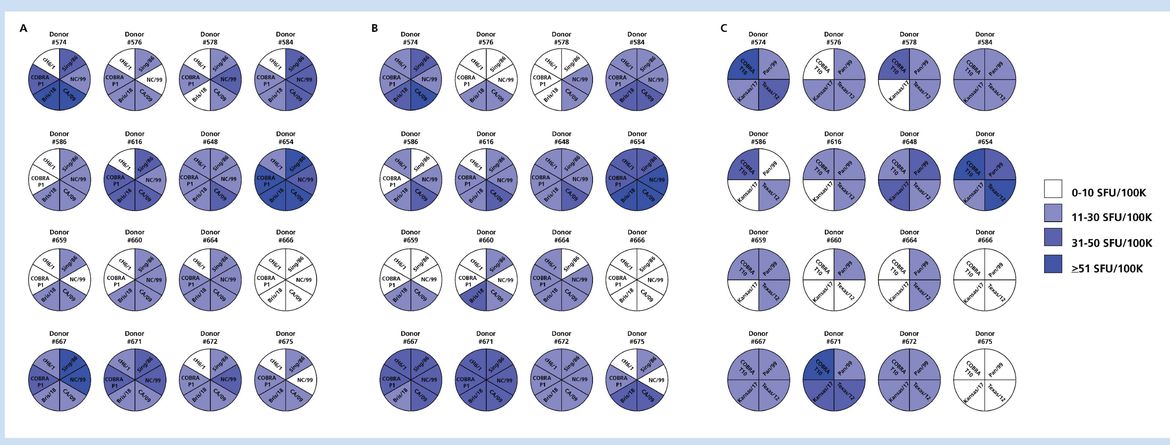

Fig. 5: High-throughput characterization of B_{mem}-derived ASC reactivity against a panel of recombinant HA (rHA) proteins. (A-C) Pie chart representation of B_{mem}-derived IgG^+ ASC reactivity of PBMC donors (n=16) against a panel of rHA proteins. (A) Visualization of B_{mem}-derived IgG^+ ASC breadth against rHA proteins representing H1N1 influenza virus strains (refer to Fig. 4 for strain abbreviations) or universal influenza vaccine candidates (COBRA P1 or cH6/1) that were absorbed to the assay membrane through affinity capture coating (ACC). (B) Visualization of B_{mem}-derived IgG^+ ASC breadth against a rHA protein panel (refer to Panel A) using an inverted assay approach. (C) Visualization of B_{mem}-derived IgG^+ ASC breadth against rHA proteins representing H3N2 influenza virus strains, or the COBRA T10 universal influenza vaccine candidate, that were absorbed to the assay membrane through ACC. Abbreviations of H3N2 viruses are as follows; A/Panama/2007/1999 = Pan/99, A/Texas/50/2012 – Texas/12, A/Kansas/14/2017 = Kansas/17. COBRA T10 rHA represents a universal vaccine candidate that was generously provided by the laboratory of Ted M. Ross and has been described previously (PMID 28978710). Legend depicting magnitude of B_{mem}-derived IgG^+ ASC response (enumerated as spot-forming units = SFU) using shades of blue applies to all panels.

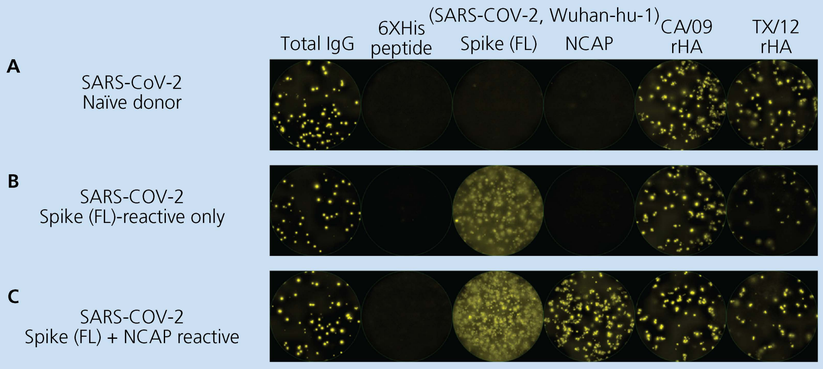

Fig. 2: Categorization of post-COVID era donors based on distinct B_{mem} immunophenotypes against SARS-CoV-2 Spike (FL) and NCAP proteins. (A-C) Representative well images depicting B_{mem}-derived IgG^+ antibody-secreting cell (ASC) activity of donor PBMC following pre-stimulation irrespective of antigen-specificity (total, ~937 cell input), or against recombinant proteins (~3 x 10^5 cell input) representing SARS-CoV-2 (Spike or NCAP) or seasonal influenza hemagglutinin (rHA) (CA/09 = H1N1, TX/12 = H3N2). (A) Representative well images depicting a donor categorized as SARS-CoV-2 naïve based on the absence of B_{mem}-derived IgG^+ ASC activity against Spike (FL) and NCAP. (B) Representative well images depicting a donor categorized as Spike (FL)-reactive only based on B_{mem}-derived IgG^+ ASC reactivity. (C) Representative well images depicting a donor categorized as Spike (FL) and NCAP-reactive based on B_{mem}-derived IgG^+ ASC reactivity. Contrast enhancements were uniformly performed on all images to aid their visualization.

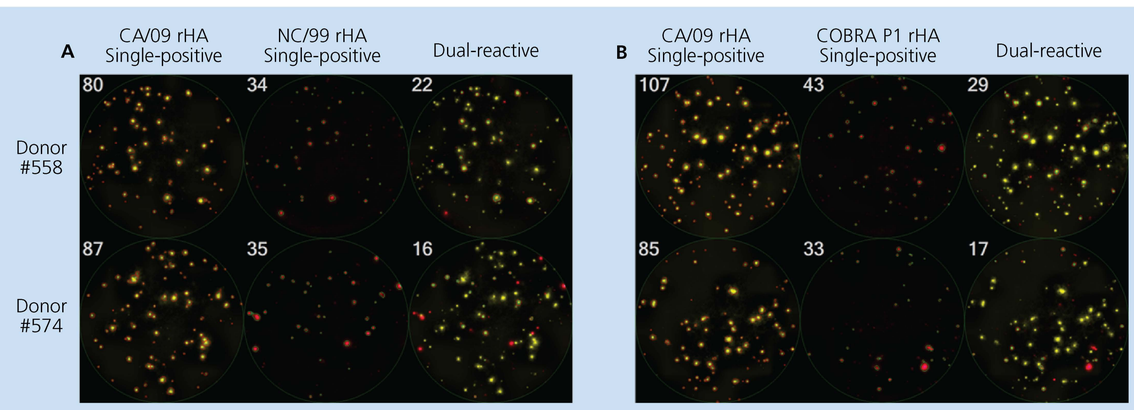

Fig. 6: Assessment of B_{mem}-derived ASC cross-reactivity with antigenically distinct rHA probes. (A and B) Representative well images depicting B_{mem}-derived ASC reactivity of two donors (#558 and #574) PBMC against rHA proteins (~1 x 10^5 cell input) using an inverted assay approach and fluorescently-labeled probes. (A) Number of Bmem-derived ASC secretory footprints that retained fluorescently-labeled CA/09 rHA or NC/99 rHA probes, in addition to secretory footprints that were dual-reactive with both rHA probes, are indicated in the upper left corner of the respective well images. (B) Number of B_{mem}-derived ASC secretory footprints that retained fluorescently-labeled CA/09 rHA or COBRA P1 rHA probes, in addition to secretory footprints that were dual-reactive with both rHA probes, are indicated in the upper left corner of the respective well images. Contrast enhancements of well images were performed to aid their visualization.

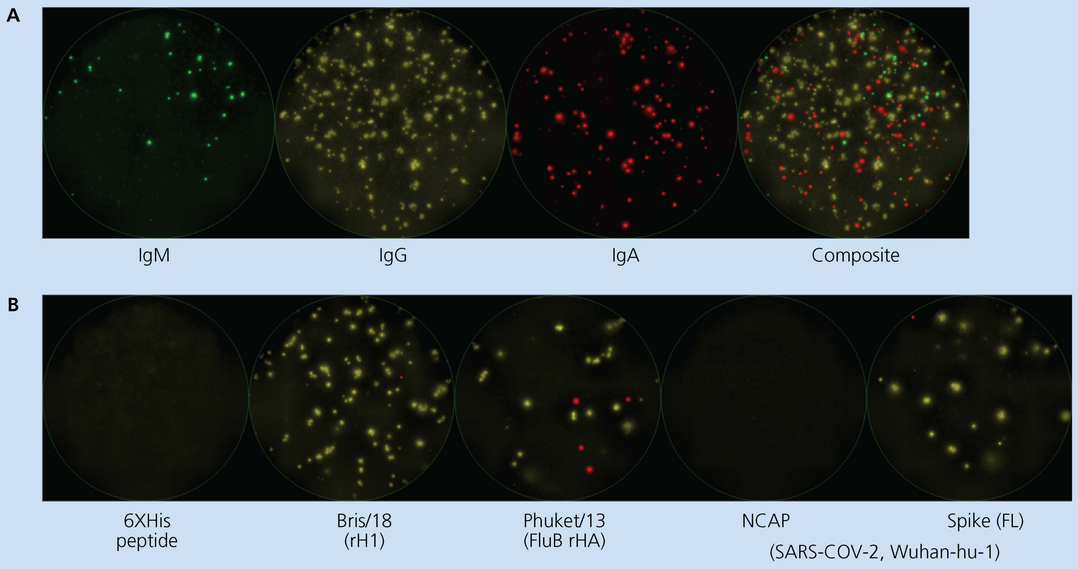

Fig. 3: Assessment of antibody-secreting cell (ASC) reactivity acutely following seasonal influenza and COVID-19 mRNA vaccination. (A) Multiplexed FluoroSpot images depicting ASC activity, irrespective of antigen specificity, present directly *ex vivo* in peripheral blood mononuclear cells (PBMC) (~3 x 10^5 cell input) collected eight days following vaccination with inactivated influenza virus (IIV) and COVID-19 mRNA. Images of the individual color planes enabling detection of IgM^+ (green), IgG^+ (yellow) or IgA^+ (red) ASC, in addition to a virtual composite images in which the respective color planes are super-imposed, are shown. (B) Assessment of IgA^+ and IgG^+ ASC reactivity against recombinant hemagglutinin (rHA) proteins representative of influenza A H1N1 (A/Brisbane/02/2018 = Bris/18) or influenza B (B/Phuket/3073/2013 = Phuket/13) vaccine strains, or NCAP or Spike (FL) proteins representative of the Wuhan-Hu-1 strain of SARS-CoV-2. All recombinant proteins possessed a C-terminal hexahistidine (6XHis) tag and were coated on the assay membrane through affinity capture coating. Contrast enhancements were performed on all images to aid their visualization.

ePBMC®

Human B cell

Mouse B cell

ImmunoSpot® By C.T.L.

Conflict of interest. P.V.L. is Founder, President, and CEO of Cellular Technology Limited (CTL), a company that specializes in immune monitoring by ELISPOT. G.A.K., N.B., L.Y. and Z.L. are employees of CTL. C.W. and S.K. declare no conflict of interest.