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Multiplex analysis of antigen-specific memory B cells in humans using reversed B-cell FluoroSpot

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ABSTRACT

Analysis of B-cell specificities at the single cell level provides important information on how the B-cell compartment responds when challenged by infection or vaccination. We recently developed a reversed B-cell FluoroSpot assay and showed that it could be used to detect B cells specific for different antigens simultaneously in a mouse model. The aim of this study was to further develop the method to detect and quantify antigen-specific memory B cells (MBCs) in humans where circulating antigen-specific cells are less frequent. We show that MBCs specific for three antigens, tetanus toxoid, hepatitis B surface antigen and cytomegalovirus pp65, could be detected simultaneously in one well. In addition to enumerating antigen-specific MBCs, we also assessed the spot volume to estimate the intensity of the response in individual cells and found this to be a new and sensitive approach to study MBC responses after vaccination. This unique B-cell FluoroSpot approach provides a simple and sensitive multiplex analysis of MBCs and can be adapted to most antigens and host species.

1. Introduction

Studies on circulating antigen-specific memory B cells (MBCs) can provide detailed insights on the development of immune responses after exposure to infection or vaccination (Crotty et al., 2004). Since MBCs can persist for a very long time and can, upon reactivation, mount an antibody response decades after the first encounter with an antigen, these cells can provide important information regarding past exposure (Crotty et al., 2003). This is particularly important since some studies have shown that circulating antibodies can drop to undetectable levels after vaccination or infection while retaining circulating antigen-specific MBCs (Bauer and Jilg, 2006; Ndungu et al., 2012; Latner et al., 2011). Therefore, studies on MBCs can be highly informative regarding the antigen-specific immune response. However, the very low frequencies of these cells in peripheral blood make the analysis of antigen-specific MBCs challenging and thus requiring highly sensitive methods (Lanzavecchia et al., 2006).

The B-cell ELISpot assay is an established technique for the analysis of antigen-specific MBCs at the single cell level that was described already in 1983 (Czerkinsky et al., 1983). The robustness and sensitivity of the ELISpot makes it a valuable tool to study B-cell responses and the method has been used for studies of MBC responses to many pathogens (Ndungu et al., 2013; Kesa et al., 2012; Weinberg et al., 2018). ELISpot is however restricted to the analysis of only one antigen per well, limiting the number of antigens that can be studied due to blood volumes available, especially in samples from children. The B-cell FluoroSpot assay, an advanced variant of ELISpot, allows for multiplex analysis of several analytes at the single cell level since it uses fluorescent detection systems rather than a precipitating substrate (Gazagne et al., 2003). Recently, we described a reversed B-cell FluoroSpot assay using tagged antigens to detect B cells that were reactive with either of up to four different antigens simultaneously (Jahnmatz et al., 2016). The definition "reversed" is used since, instead of coating the well with an antigen, the most common approach for antigen-specific B-cell FluoroSpot/ELISpot, wells are coated with antibodies that capture antibodies secreted by the B cells. Detection of antigen-specific B-cells is then achieved by using peptide-tagged antigens followed by fluorescently labeled anti-tag reagents. The assay was evaluated using mouse hybridomas and splenocytes from hyperimmunized mice. Here, we

Abbreviations: BSA, bovine serum albumin; CMV.pp65, cytomegalovirus pp65; FBS, fetal bovine serum; HBsAg-S, hepatitis B surface antigen small; mAb, monoclonal antibody; MBCs, memory B cells; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; RT, room temperature; TT, tetanus toxoid

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developed the assay further for multiplex analysis of antigen-specific MBCs in human peripheral blood mononuclear cells (PBMC), where antigen-specific cells are relatively rare in circulation.

2. Materials and methods

2.1. Study population

Buffy coats from anonymous blood donors (n = 23) were received from the Blood Bank at Karolinska University Hospital, Stockholm, Sweden. Moreover, six individuals scheduled for Engerix B™ (GlaxoSmithKlein, Rixensart, Belgium) vaccination were recruited to the study. Each dose of the vaccine contained 10 μg/0.5 mL Hepatitis B surface antigen (HBsAg). Participants filled in a questionnaire regarding vaccination history. Five of the individuals were to receive their first vaccination, while one individual was vaccinated with two doses 12 years earlier. After informed consent, blood samples were collected before and 18–21 days following vaccination.

2.2. Preparation and stimulation of cells

PBMCs were isolated from buffy coats and whole blood collected in EDTA tubes through density gradient centrifugation with Ficoll-Paque Plus according to instructions from the manufacturer (GE Healthcare, Uppsala, Sweden). After washing in phosphate buffered saline (PBS), cells were diluted in fetal bovine serum (FBS) (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% dimethyl sulfoxide (Sigma-Aldrich, Saint Louis, MO, USA) and stored in liquid nitrogen. Recovered PBMC were cultivated in Roswell Park Memorial Institute 1640 media (Invitrogen Life Technologies) supplemented with 10% FBS, 100 U/mL penicillin, 10 mM HEPES and 100 μg/mL streptomycin (all from Invitrogen, Life Technologies) (culture medium). To allow quiescent MBCs to differentiate into antibody-producing plasmablasts, the cells were pre-stimulated with 1 μg/mL R848 and 10 ng/mL recombinant human Interleukin-2 (both from Mabtech, Nacka Strand, Sweden) for five days at 37 °C in 5% CO_2. Before use in the FluoroSpot, cells were counted and analyzed for viability using a Muse® Cell Analyzer (Merck, Darmstadt, Germany).

2.3. Production of tagged antigens

Recombinant HBsAg (UniProt accession number Q773S4) and CMV.pp65 (P06725) were produced in a mammalian expression system as previously described (Jahnmatz et al., 2016). Briefly, pcDNA3.1/Zeo (−) (Thermo Fischer Scientific, Waltham, MS, USA) plasmids containing the gene sequences for the antigen as well as the tag were transiently transfected into Expi293F cells using the Expi293 Expression system according to the instructions by the manufacturer (Thermo Fischer Scientific). The transfected cells were then cultivated for 6 days before supernatants were harvested and stored in liquid nitrogen until use. HBsAg was tagged N-terminally with the peptide tag BAM (amino acid sequence DAEFRHDSGY), and CMV.pp65 with a GAL tag (YPGQAPPGAYPGQAPPGA) at the C terminus (Jahnmatz et al., 2016). Purified TT (Statens Serum Institut, Copenhagen, Denmark) was biotinylated using long-chain biotinyl-N-hydroxysuccinimide ester acid (Thermo Fisher Scientific) according to the manufacturer's instructions.

2.4. Verification of protein expression and determination of antigen concentration

Protein expression was verified by SDS-Page and Western Blot for TT and CMV.pp65 (Supplementary Fig. 1A), and by ELISA for HBsAg (Supplementary Fig. 1C). While the concentration of TT was known, the protein concentration of CMV.pp65 was semi-quantified using Western Blot with anti-tag reagents, followed by band intensity analysis using ImageJ software (Heidebrecht et al., 2009) (Supplementary Fig. 1B). The concentration of HBsAg was determined by electrochemiluminescence method (ECLIA) using the HBsAg II quant II e801 test (Roche Diagnostics GmbH, Mannheim, Germany) by certified personnel at Karolinska University Hospital Laboratories, Stockholm, Sweden (Supplementary Fig. 1D).

2.5. Reversed B-cell FluoroSpot assay

Low fluorescent 96-well polyvinylidene fluoride plates (Merck Millipore, Burlington, MS, USA) were pre-wetted with 20 μL/well 35% ethanol and washed five times with 200 μL/well sterile H_2O. One hundred μL of mouse monoclonal antibody (mAb) anti-human IgG (MT91/145) were added to the wells at 15 μg/mL in sterile PBS and incubated overnight (18–24 h) at 4 °C. The plates were washed five times with sterile PBS (200 μL/well) and blocked with 200 μL/well culture medium for 1 h at 37 °C. After that, 100 μL/well of culture medium containing 500,000 pre-stimulated cells was added to each well in duplicates, followed by overnight incubation at 37 °C in 5% CO_2. The optimal amount of cells was defined in pilot tests (Supplementary Fig. 2A). After incubation, the plates were washed five times with sterile PBS (200 μL/well). To detect antigen-specific MBCs, 100 μL/well of biotinylated TT diluted to 1 μg/mL and/or peptide-tagged antigens HBsAg-S diluted to 1890 IU/mL and/or CMV.pp65 diluted to 5 μg/mL in PBS with 0.1% bovine serum albumin (BSA) (PBS/BSA) was added to each well and incubated for 1 h at room temperature (RT). The optimal concentration of each antigen was defined in pilot experiments using serial dilutions (Supplementary Fig. 2B). After another wash cycle, 100 μl of fluorophore-conjugated detection reagents (mAb anti-GAL-490, mAb anti-BAM-640 and/or Streptavidin-550) diluted to 0.5 μg/mL in PBS with 0.1% BSA and 1% human plasma were added to each well and incubated for 1 h. Human plasma was added to block potential binding of coating mAb to anti-tag detection mAb. The frequency of total IgG-producing cells was analyzed separately using 25,000 pre-stimulated PBMCs in duplicate wells. In order to detect IgG-producing cells, 100 μL/well of biotinylated mAb anti-human IgG (MT78/145-biotin) diluted to 0.5 μg/mL, followed by Streptavidin-550 in PBS/BSA, were used for detection. Plates were again washed and incubated for 15 min at RT with 50 μL fluorescent enhancer in each well. The enhancer was then removed thoroughly by emptying the liquid rapidly from the wells, the plate underdrain discarded, and the plates were left to dry while protected from light. All reagents were from Mabtech (Nacka Strand, Sweden). Plates were analyzed using FluoroSpot reading systems iSpot Spectrum with software version 7 (built 14,790) (AID, Strassberg, Germany) or Mabtech IRIS™ FluoroSpot reader with Apex™ software version 1, in which fluorescent spots were counted using separate wavelength-specific filters. The filters used were for FITC, Cy3 and Cy5 enabling detection of fluorophores absorbing and emitting light at 490/520, 550/570 and 640/660 nm, respectively. As negative control for indication of non-specific spots, stimulated PBMCs (500,000 cells) were treated as above and detected in a separate well using irrelevant antigens (cow-, woodchuck and cat Interferon gamma) tagged similarly as HBsAg, CMV.pp65 or TT, as described previously (Jahnmatz et al., 2016).

2.6. Serology

The levels of anti-HBs antibodies in plasma samples were measured by ECLIA using the Cobas 6000 analyzer (Roche Diagnostics, Rotkreuz ZG, Switzerland) by certified personnel at Karolinska University Hospital Laboratories, Stockholm, Sweden. An anti-HBsAg level above 10 mIU/mL was set as threshold for seropositivity, based on protective titers (Jack et al., 1999).

3. Results and discussion

3.1. Characterization and development of tagged antigens

The antigens used in the B-cell FluoroSpot assay, TT, HBsAg and CMV.pp65, were selected since responses against these antigens are expected to be frequent within the population. Tetanus vaccination gives rise to long-lasting immune memory (Hammarlund et al., 2016)

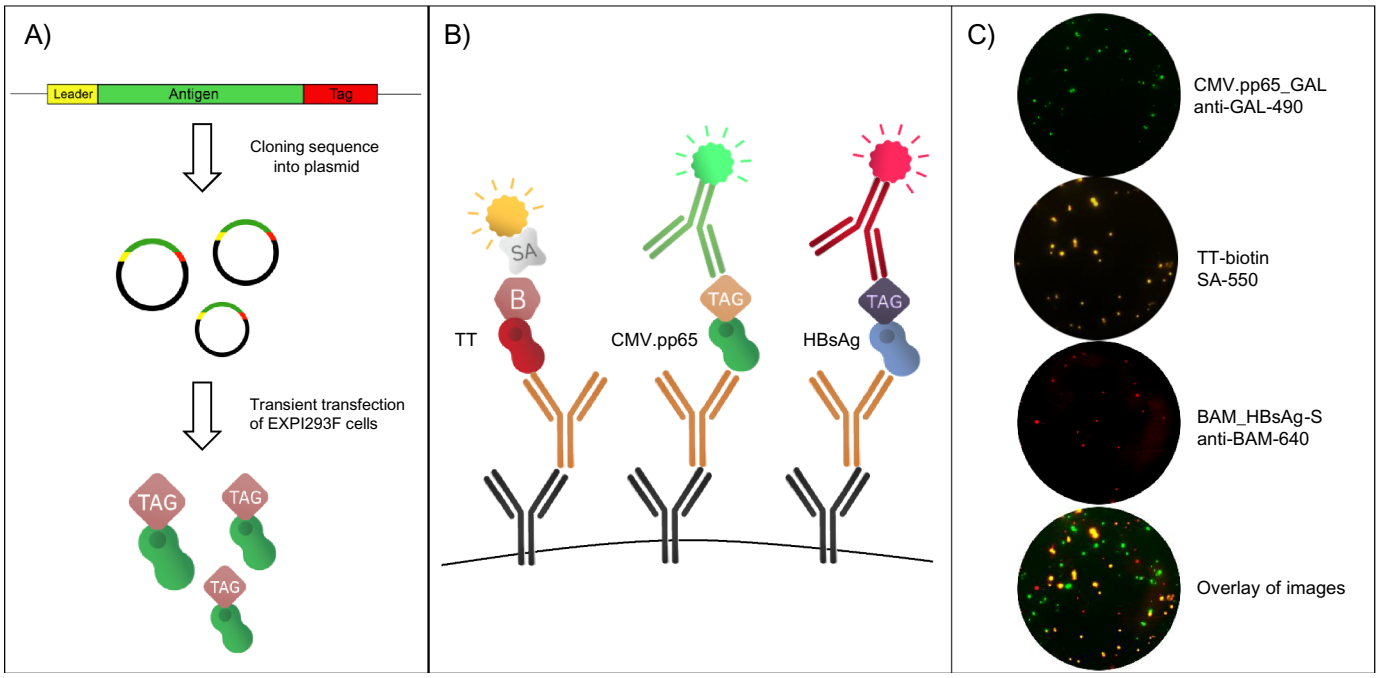

Fig. 1. Principle of the multiplex B cell FluoroSpot assay. A) Schematic presentation of antigen constructs and procedure to produce recombinant tagged antigens in mammalian cells. B) Assay principle of the B-cell FluoroSpot used for the analysis of antigen-specific B cells. Immobilized anti-human IgG antibodies are used to capture IgG secreted by B cells. IgG secreted by B cells is then allowed to bind antigens labeled with biotin or peptide tag, respectively. By finally using fluorescently labeled detection systems, i.e. streptavidin (SA) or different antibodies specific for the tags, the position of antigen-specific B cells can be detected as a spot on the membrane. C) Representative B-cell FluoroSpot readout donor from a single experiment (buffy coat donor 2, see Fig. 2) reactive against HBsAg, CMV.pp65 and TT. Well images for each antigen, as well as a computerized overlay of the three separate images, analyzed using the iSpot Spectrum FluoroSpot reader (AID, Strassberg, Germany).

and also hepatitis B vaccination can elicit detectable MBCs after full vaccination (Tian et al., 2018). CMV is a persistent virus with a prevalence of approximately 80% in Europe (La Rosa and Diamond, 2012).

Antigens used in the FluoroSpot can be tagged recombinantly or chemically. Chemical labeling may have some disadvantages. For example, biotinylation with active ester reagents will target any lysine side chains or the amino acid terminal, and it is difficult to target the tag to one given position on the antigen. Moreover, biotinylation of many sites on the antigen may affect its antigenicity and the ability of antibodies to recognize their target (Kay et al., 2009). Ideally, one tag is coupled to each antigen molecule which is more easily achieved when antigens are tagged recombinantly. Here, we used purified TT that was chemically labeled with biotin reagent at a molar excess ensuring biotinylation primarily of one amino group per TT molecule, while HBsAg and CMV.pp65 were expressed recombinantly with different peptide tags (Jahnmatz et al., 2016). These antigens were expressed by transfecting Expi293F cells with a plasmid encoding the antigen sequence linked to the tag sequence (Fig. 1A). The protein concentration of CMV.pp65 and HBsAg was then determined using Western Blot/ImageJ and ECLIA respectively (Supplementary Fig. 1). All antigens were then used directly in the FluoroSpot assay after optimizing the concentrations of the respective antigens (Supplementary Fig. 2). The optimal concentration for the antigens in FluoroSpot was 1890 IU/mL for HBsAg, 5 μg/mL for CMV.pp65 and 1 μg/mL for TT. The principle and set up of the assay for the detection of antigen-specific B cells are described in Fig. 1B. As shown in Fig. 1C, antigen-specific MBCs against all three antigens could be detected simultaneously in a single well with cells from an individual donor. However, not all individuals displayed a response to all antigens.

3.2. Analysis of multiple MBC antigen-specificities using the FluoroSpot assay

The B-cell FluoroSpot was used to enumerate antigen-specific MBCs specific for HBsAg, TT or CMV.pp65 in PBMC from 23 anonymized blood donors (Fig. 2). In parallel, background responses to irrelevant antigens labeled with the corresponding tags was measured; the number of spots against irrelevant control antigens ranged between 0 and 4 spots per well (Fig. 2). Also, the number of total IgG-secreting B cells was determined for each sample using an anti-IgG detection mAb instead of tagged antigen and anti-tag detection reagents (Fig. 2).

In order to account for overall B-cell expansion after stimulation, MBC reactivity was calculated as the proportion of antigen-specific MBCs per total IgG-secreting MBCs. For TT, the median proportion of MBCs/total IgG was 0.14% with a range of 0–2.25%. For CMV.pp65 the median was 0.03% with a range of 0–0.91%, and for HBsAg the median was 0.07% with a range of 0–0.38% (Fig. 3). The responses varied between donors, but most donors displayed a reactivity to TT and HBsAg above 0.01% antigen-specific MBCs. For CMV, 15 donors displayed a response above 0.01% antigen-specific MBCs whereas the other 8 donors had lower responses comparable to those obtained with irrelevant background controls (Fig. 3). Measurements of corresponding antigen-specific antibody levels in plasma could not be performed because only PBMCs were isolated from buffy coats from these anonymous blood donors.

The FluoroSpot reader analyzes each antigen specificity separately using specific filters for each antigen/fluorophore (Janetzki et al., 2014). When assembling the three images from each well, no spots were co-positioned i.e. each spot represented one B cell with one specificity. Adding the antigens in separate wells or together in a single well did not affect the frequency of counted spots (Fig. 4). When evaluating the number of cells needed for the analysis, the number of spots was reduced by approximately half when 250,000 cells instead of 500,000 were used. A similar reduction in spot number could be observed when cell numbers were reduced to 125,000. To avoid detection of antigen-specific MBCs at too low numbers, 500,000 cells/well was later used (Supplementary Fig. 2A).

Furthermore, no spots were detected hence not counted in control wells containing either no cells (Supplementary Fig. 2C) or no coating antibody (Supplementary Fig. 2D).

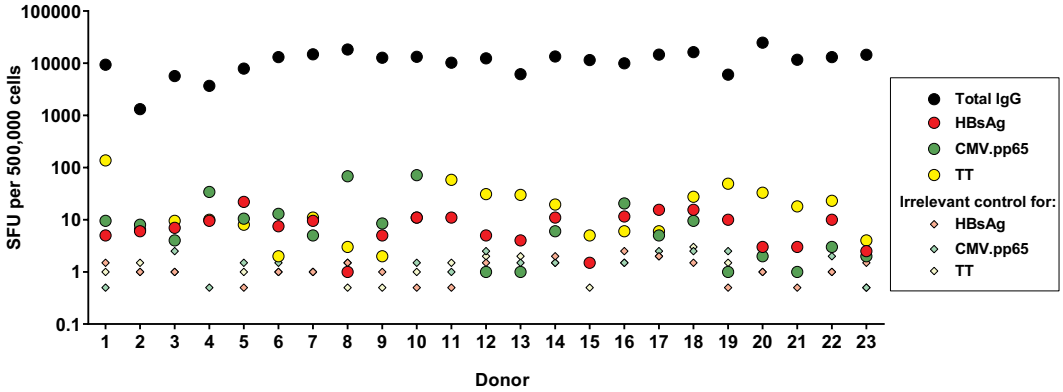

Fig. 2. B-cell FluoroSpot analysis of antigen-specific B cells against HBsAg, CMV.pp65 and TT in 23 donors with unknown vaccination history. On the Y-axis, the number of antigen-specific spots and number of total IgG spots counted per 500,000 cells are shown. The red dot shows the number of spots of HBsAg-specific B cells from each donor, the green dot CMV.pp65 and the yellow dot TT. The diamond symbols show the number of spots of B cells obtained with irrelevant control antigen tagged in the same way as HBsAg (cow Interferon gamma; pale red), CMV.pp65 (woodchuck Interferon gamma; pale green) and TT (cat Interferon gamma; pale yellow). The numbers of Total IgG spots are shown as black dots. Data pooled from four separate experiments with n = 5–6 donors in each experiment. Plates were analyzed using the iSpot Spectrum FluoroSpot reader (AID, Strassberg, Germany). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

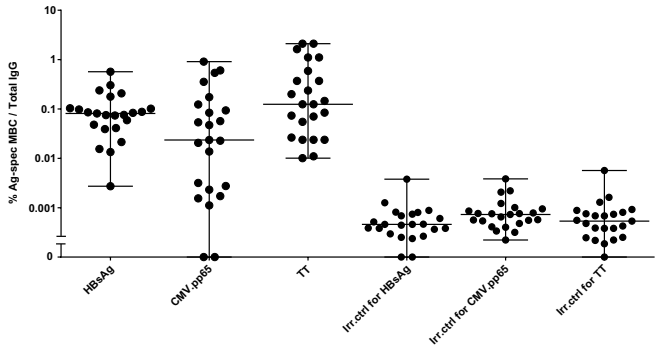

Fig. 3. B-cell FluoroSpot analysis of antigen-specific B cells against HBsAg, CMV.pp65 and TT in 23 donors with unknown vaccination history. On the Y-axis, the percentage of antigen-specific B cells from each donor responding against an antigen and the percentage of B cells from each donor responding to an irrelevant control antigen tagged similarly to HBsAg (cow Interferon gamma), CMV.pp65 (woodchuck Interferon gamma) or TT (cat Interferon gamma) are shown as dots. The percentage of antigen-specific MBCs was calculated by dividing the number of antigen-specific spots with the number of total IgG spots counted. Lines represent median and range of responses against each antigen from all donors. Data shown was pooled from four separate experiments with n = 5–6 donors in each experiment. Plates analyzed using the iSpot Spectrum FluoroSpot reader (AID, Strassberg, Germany).

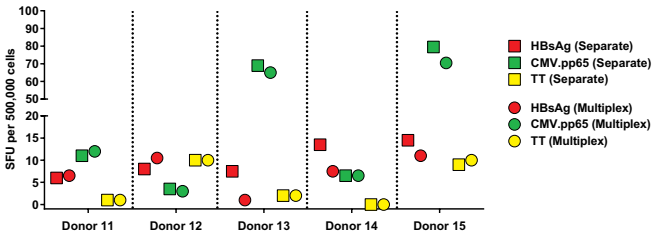

Fig. 4. Analysis of number of antigen-specific B cells in five donors using the B-cell FluoroSpot assay with separate wells for each antigen or by multiplex analysis. On the Y-axis, the number of antigen-specific spot-forming units (SFU) counted per 500,000 cells are shown. Square symbols represent spots from MBCs reactive with HBsAg (red), CMV.pp65 (green) and TT (yellow) obtained with antigens tested in separate wells. Circle symbols represent spots from MBCs reactive with HBsAg (red), CMV.pp65 (green) and TT (yellow) obtained with antigens tested together in a well. Plates were analyzed using the iSpot Spectrum FluoroSpot reader (AID, Strassberg, Germany). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Since it is well recognized that TT vaccination is a potent inducer of long-lived MBCs (Hammarlund et al., 2016; Buisman et al., 2009), it was not surprising that this antigen had the highest proportion of antigen-specific MBCs among the donors. These results are in line with the results of a similar study using ELISpot (Lee et al., 2011). We also observed that the proportions of antigen-specific MBCs against HBsAg were generally low for all of the donors tested. Since these donors were anonymous, we could not correlate the results to vaccination history. Some donors might have been previously vaccinated; however, it was not surprising that several donors did not have MBC responses against HBsAg since hepatitis B vaccination has not been part of the national immunization program in Sweden. As for CMV, the frequency of subjects responding to pp65, with higher responses than to the irrelevant control, was in line with a recent study showing a seroprevalence against CMV in Sweden of 83% (Olsson et al., 2017). Regarding the magnitude of the response, in responding subjects, it may be that stronger response (in terms of magnitude) could have been obtained using another antigen than pp65, such as e.g. glycoprotein B, an antigen shown to be an important target for antibodies (Potzsch et al., 2011).

3.3. HBsAg-specific MBCs before and after vaccination

In order to measure MBCs before and 18–21 days after HBV vaccination, PBMC and plasma samples from six donors were tested in the reversed B-cell FluoroSpot assay and for circulating anti-HBs antibody levels, respectively. Before vaccination, one study participants (participant 5) displayed a response of 0.24% HBsAg-specific MBCs of total IgG-secreting MBCs, whereas the others had no or very low levels of HBsAg-specific MBCs (0–0.03% MBCs/Total IgG) (Fig. 5A), with only 1–2 spots per well similar to responses to irrelevant antigens (data not shown). Participant 5, unlike the other participants, had been vaccinated and boosted against HBV 12 years earlier.

According to antibody levels measured before vaccination, none of the participants were seropositive for HBsAg, whereas after vaccination, three participants had seroconverted (Fig. 5B). The highest increase in antibody levels was seen in participant 5 who was vaccinated previously (from 5.25 to > 1000 mIU/mL). When the proportion of HBsAg-specific MBCs after vaccination was measured in FluoroSpot, again, only the previously vaccinated donor showed measurable MBC reactivity and displayed a three-fold increase in the proportion of HBsAg-specific MBCs (Fig. 5C). A likely reason for this could be that a primary vaccination against Hepatitis B is not sufficient to induce detectable MBCs in circulation. Alternatively it may be that the time it

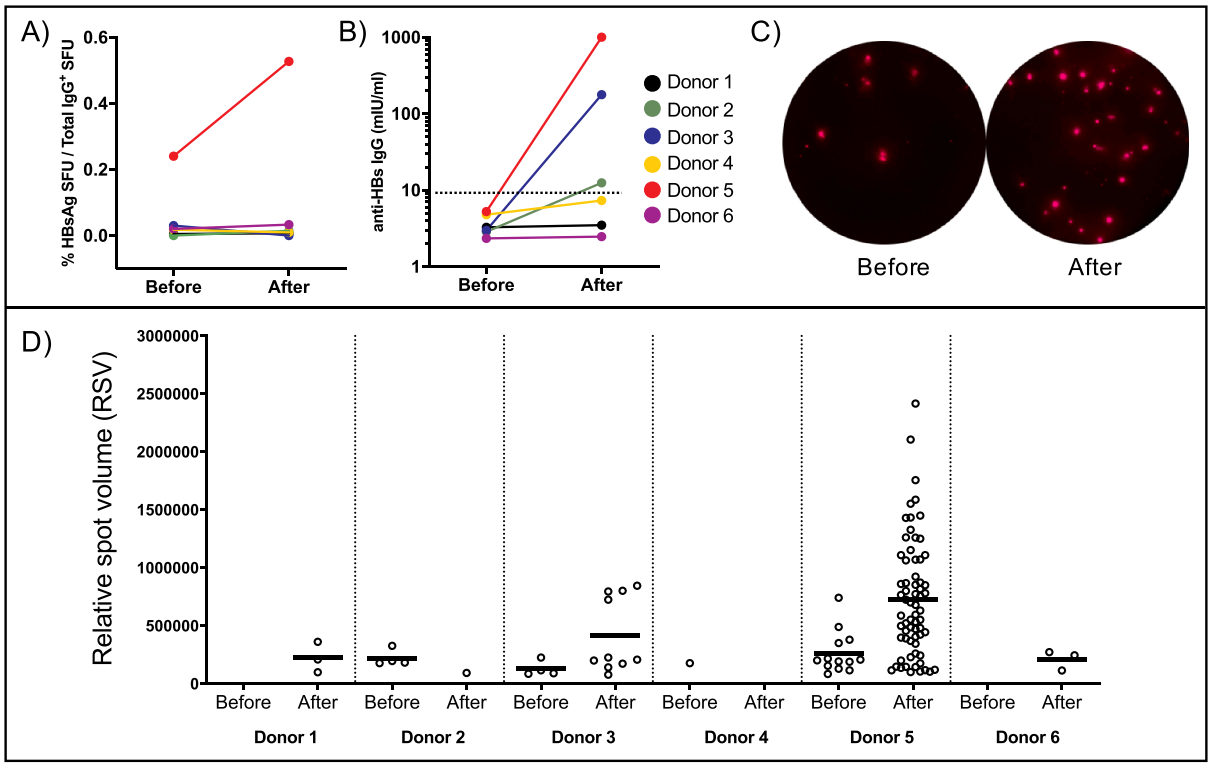

Fig. 5. FluoroSpot and serology analyses of antigen-specific B cells and antibody levels before and after hepatitis B vaccination. A) B-cell reactivity against tagged HBsAg before and after hepatitis B vaccination was measured by FluoroSpot. On the Y-axis, the percentage of antigen-specific B cells per total number of total IgG spots is shown. B) Plasma antibody levels against HBsAg before and after hepatitis B vaccination as measured by electrochemiluminescence method (ECLIA) from a single experiment with all donors. Values are shown in mIU/mL and dashed border indicates threshold considered for seropositivity (10 mIU/mL). C) Images showing FluoroSpot readout of HBsAg specific B cells before and after vaccination against hepatitis B, in the only responsive donor. D) Relative spot volume (RSV) of each HBsAg specific spot from donors before and after vaccination. On the Y-axis, the RSV value is shown. RSV value of each individual spots from duplicates wells are shown before and after vaccination from each donor. The mean RSV value of all spots is indicated by the black bar. Representative data from two repeated experiments with all 6 donors is shown. Plates analyzed using the Mabtech IRIS™ FluoroSpot reader (Mabtech AB, Nacka Strand, Sweden).

takes to develop detectable frequencies of MBCs in circulation is longer than the follow up time of 18–21 days, while the increase in detectable antibody levels is faster. It could also be that MBCs, despite a serological response, are so few that no significant response is detected. This slow acquisition of detectable MBCs after a primary vaccination against Hepatitis B has also been reported in another study (Galson et al., 2016). Here, it was shown that detectable MBCs could be found first after a second administration of the vaccine.

For the B-cell FluoroSpot assay performed on the vaccinated individuals, the Mabtech IRIS™ FluoroSpot reader was used. In addition to counting the number of spots, this reader also provides data on the size and fluorescent intensity of each spot and based on these variables calculates a relative spot volume (RSV) (Pla and Jalden, 2018). The RSV reflects the relative amount of antibody produced by a B cell but also the affinity of the antibody. Interestingly, when the RSV value was analyzed for HBsAg spots for each donor before and after vaccination, a small increase in mean RSV could be observed after vaccination in two of the donors that had seroconverted without having any detectable increase in spot frequency (Fig. 5D). These results suggest that changes in RSV values before and after vaccination could reveal other details compared to only counting the spot numbers, even though additional studies are needed to shed more light on this. Furthermore, by using the reversed B-cell FluoroSpot, our results show that HBsAg-specific MBCs can be detected in a seronegative donor who was vaccinated 12 years previously. Although restricted to a limited number of individuals, these results support previous studies demonstrating that vaccinated individuals with antibody levels below the protective threshold of 10 mIU/ml, can still harbor circulating HBsAg specific MBCs (Bauer and Jilg, 2006).

3.4. Adaptability of the reversed B-cell FluoroSpot using the tag/anti-tag approach

The B-cell FluoroSpot was here designed for three well-characterized antigens. The tag/anti-tag detection systems can potentially be adapted for most antigens. Multiplex analysis of B cells is highly relevant in many scenarios, especially when evaluating immune responses to multicomponent or polyvalent vaccines containing multiple antigens or as previously mentioned, when the amount of material is limited, such as in samples from children.

There are a number of advanced techniques for single B-cell analysis such as flow cytometry, microfluidic trapping and mass cytometry, and several more are emerging (Chattopadhyay et al., 2014). These methods allow in-depth analysis of single B cells in a heterogenic cell population and some of them also enable the possibility to isolate single cells for further analysis. The advantages of the multiplex B-cell FluoroSpot compared to other techniques for analyzing antigen-specificity at the single cell level, is the adaptability, higher sensitivity and relatively low cost. The reversed B-cell FluoroSpot has been used to study human B-cell responses against dengue virus (Hadjilaou et al., 2015; Adam et al., 2018) and in transplantation medicine (Luque et al., 2018) but the methodological design with fluorescently labeled antigen or antigen-specific reagents used in those studies, increases the potential risk of blocking important epitopes with the fluorophore, and limits the use of the method to one given antigen. The use of a tag/anti-tag approach used here expands the adaptability and multiplex possibilities of the FluoroSpot assay and facilitates different types of studies on B-cell responses after infection or vaccination.

4. Concluding remarks

We have shown that a flexible tag/anti-tag system can be used to simultaneously detect separate antigen-specific MBCs in humans with a reversed B-cell FluoroSpot assay. The adaptability of this assay for multiplex analysis at the single cell level will be a valuable tool for evaluation of vaccines and other research areas such as exploration of immunological memory to natural infections after exposure and for diagnosis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2019.112715.

Ethics

Ethical approval was obtained from the Regional Ethical Board in Stockholm, Sweden (2017/2228-31). Informed consent was given by all study participants.

Declaration of Competing Interest

Peter Jahnmatz, Bartek Makower and Niklas Ahlborg are employed by Mabtech AB, Sweden. Several of the reagents and one of the FluoroSpot reader systems used in this study are produced by Mabtech.

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