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Peak Frequencies of Circulating Human Influenza-specific Antibody Secreting Cells Correlate with Serum Antibody Response after Immunization

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Abstract

Upon vaccination, B cells differentiate into antibody secreting cells (ASCs) that migrate via the circulation to tissues. The kinetics of this response and the relationship of circulating ASCs to protective antibody titers have not been completely explored.

Methods—Influenza-specific and total-IgG ASCs were enumerated by Elispot and flow cytometry daily in the blood in 6 healthy adults after trivalent influenza vaccination (TIV).

Results—Peak H1-specific IgG ASC frequencies occurred variably from day 5 to 8 and correlated with the fold-rise rise in hemagglutination inhibition (HAI titers); $r=0.91$, $p=0.006$. H3-specific IgG ASC frequencies correlated less well, perhaps due to a mismatch of the H3 protein in the vaccine and that used in the Elispot assay. Peak frequencies of vaccine-specific and total-IgG ASCs were 0.3% and 0.8%, respectively, of peripheral blood mononuclear cells (PBMC). Peak TIV-, H1-, H3-, and total-IgG ASC frequencies were 1736 ± 1133 , 626 ± 520 , 592 ± 463 , and 4091 ± 2019 spots/ 10^6 PBMC, respectively. Peak TIV-, H1-, and H3-specific IgG ASC of total-IgG ASC frequencies constituted $63\% \pm 21$, $26\% \pm 10$, $22\% \pm 17$, respectively.

Conclusion—After immunization with inactivated influenza vaccine the peak in influenza-specific ASC frequencies is variable but correlates well with the magnitude of protective HAI responses.

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Keywords

Antibody secreting cells; plasmablasts; influenza

1. Introduction

In healthy adults, total-IgG antibody secreting cells (ASCs) with unknown antigen specificity circulate in relatively low frequencies of 250-300/million PBMCs at steady state [1]. Upon antigen exposure during vaccination or infection, a massive expansion of IgG ASCs burst into the blood circulation as they transit to bone marrow or tissue sites of inflammation [2]. The result is a subsequent increase of antigen-specific serum antibody levels with little detectable non-specific antibodies generated [3,4]. However, the antigen-specific ASC frequencies, their kinetics, and their correlation with serum antibody levels have been largely unexplored.

Historically, antibody measured by hemagglutination inhibition (HAI) and microneutralization assays has replaced traditional neutralization assays and has been correlated with protection from infection with influenza [5]. Generation of a serum HAI titer $\geq 1:40$ one month after vaccination is commonly used as a biomarker of protection, while a 4-fold or greater rise in HAI or neutralizing titer defines seroresponders [6,7]. It is possible that measurement of ASC responses could also be used to identify responders, and perhaps more quickly than standard assays that use acute and convalescent serum samples. If so, this assay could prove useful when developing new vaccines, such as during an influenza pandemic.

Despite a likely association, a clear relationship between ASC frequencies with increases in antibody levels has not been demonstrated [8,9]. This may be due to several factors. For instance, the relationship may be obscured by the complexity of the antigenic components in the trivalent influenza vaccine, and would require correlating the response to each antigen separately. Another factor may involve individual variability of ASC kinetics since these cells are present in the circulation very transiently. Therefore, in this study, we assessed the variable magnitude and timing of circulating ASCs to the whole vaccine and to each of the influenza A hemagglutinin components of trivalent influenza vaccine (TIV).

2. METHODS

2.1 Study design and setting

Six healthy subjects, ages 19 to 32 years (mean $\pm$ SD, 25 ± 8), who had not received influenza vaccination for that current year were recruited at the University of Rochester Medical Center during winter/spring 2006-2007. Prior influenza vaccination history was obtained, as well as a history of influenza like illnesses in the recent past. An additional subject was recruited who received a tetanus vaccine, as well as 26 young healthy adults (14 men and 12 women, ages 37 ± 11 years) without history of concurrent infection or recent vaccination who served as control subjects. All procedures and methods were approved by the Research Subjects Review Board at the University of Rochester Medical Center.

2.2 Vaccine administration

Subjects were immunized by intramuscular injection with standard 2006-2007 seasonal TIV subvirion vaccine (Fluzone, Sanofi Pasteur) that contained hemagglutinin of A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004. Heparinized blood (20 ml) was obtained prior to immunization and daily thereafter for 12

days, days 14-15, and 28. Serum was also collected 6 months post-vaccination. The seventh subject received a tetanus toxoid vaccine (Sanofi Pasteur) and blood was collected prior to immunization and on days 4-10, 14 and 28. Vaccines administered in this study were given as a part of routine health care.

2.3 Laboratory and clinical investigation

Peripheral blood mononuclear cell (PBMC) Isolation—PBMC were isolated within 4 hours of sampling using sodium heparin sulfate tubes. Tubes were immediately inverted 8 – 10 times and centrifuged at 1500×g for 30 minutes at 20°C and the buffy coat layer transferred to a 50mL tube. The cells were washed 3 times (300×g, 10 min, 4°C) and viability assessed by Trypan Blue exclusion.

Hemagglutination Inhibition Assay (HAI): A standard HAI assay was performed according as previously described. Briefly, serial two fold serum dilutions were assays against influenza H1N1, H3N2, and B using strains contained in the 2006-07 TIV vaccine [10].

Antigen-specific antibody secreting cell (ASC) Elispot—The frequency of Influenza antigen-specific ASCs was measured by Elispot [11]. Briefly, 96-well Elispot plates (MAIPS4510 96 well, Millipore, Bedford, MA) were coated overnight at 4°C in a humidified chamber with the following antigens: purified hemagglutinin A (HA) proteins from H1 A/New Caledonia/20/99, H3 A/Wyoming/2/2003 (Protein Sciences Corp, Meriden, CT) at (1-3µg/mL), TIV Influenza Virus Vaccine (6µg/mL, Sanofi Pasteur Inc, Swiftwater, PA), or anti-human IgG (5µg/mL, Jackson ImmunoResearch, West Grove, PA). Antigens were diluted in sterile PBS to above concentrations. Bovine Serum Albumin 2% (BSA, MP Biomedicals, Solon, OH) in sterile PBS was used as an irrelevant antigen. Wells for analysis of the tetanus immunized subject was also coated with tetanus toxoid (1 Lf/mL Cylex Incorporated, Columbia, MD). Plates were blocked with RPMI and 8% fetal bovine serum for 2 hours and incubated at 37°C for 18-20 hours with 300,000, 100,000, or 33,333 PBMC in triplicate. For total-IgG plates, 100,000, 33,000, and 11,000 PBMC per well were added. After incubation, cells were aspirated and plates were washed with PBS with 0.1% Tween (PBST). Bound antibodies were detected with alkaline phosphatase-conjugated anti-human IgG antibody (1µg/mL, Jackson ImmunoResearch) for 2 hours and developed with VECTOR Blue, Alkaline Phosphatase Substrate Kit III (Vector Laboratories, Burlingame, CA). Spots in each well were counted using the CTL immunospot reader (Cellular Technologies Ltd). For analysis, background spots from wells without capture antigen (BSA coated wells) were subtracted from spots developed in antigen coated wells. Antigen-specific IgG ASC responses were considered positive if they were above the mean plus 4 standard deviations for each antigen using results from the 26 control subjects. During the development phase of the ASC assay, we had performed experiments in other subjects in order to determine the linearity of the ASC spot assay. It was determined that counts from wells with 20-250 spots provided the most accurate results, and in order to remain within this dynamic range for all samples we could use the 3 specified dilutions of unstimulated PBMCs in triplicate. We included data only from wells with spot numbers between 20-250 spots in our calculations.

Polychromatic flow cytometry—The following antibody panel against human markers was used to identify total plasmablasts or ASC from an aliquot of fresh PBMC from each daily sample [12] : IgD-FITC, CD3-Cy5.5PE, CD19-Cy7PE, CD38- Pacific Blue, and CD27-APC, and CD14-Alexa700 (BD Biosciences). One to two million events per sample were collected on an LSRII instrument (BD Biosciences) and analysis performed using FlowJo software (Treestar, Inc version 8.7.1). Total PBMC were gated on lymphocytes and monocytes using FSC and SSC. To exclude non-specific staining and non-B cells, CD14 and

CD3 were used. Plasmablasts were identified within the CD19⁺ B cell population as CD27^{hi}, CD38^{hi} and IgD⁻.

2.4 Statistical analysis

The Pearson correlation coefficient and Fisher's exact test was used to measure the correlation of two variables. The bootstrap method (with 2000 replicates) was used to test the significance of the correlation. All statistical analyses were implemented with SAS 9.1 (SAS Institute Inc., Cary, NC).

3. RESULTS

Total-IgG and TIV-specific ASC frequencies enumerated daily for each of the 6 TIV vaccinated subjects are shown in figure 1. BSA-specific IgG ASC frequencies were 0.3 ± 4 spots/ 10^6 PBMC and used as negative controls or background, and subtracted from either antigen-specific or total-IgG ASC frequencies. No antigen-specific ASCs were detected in the first two subjects (A, B) on days 1-3; therefore, day 1-3 post vaccination samples from the subsequent 4 subjects were not collected. Peak TIV-specific frequencies varied among subjects from days 5 to 8 depending on the individual and ranged from 324 to 3570 spots/ 10^6 spots PBMC (mean $\pm$ SD, $1736 \pm 1133/10^6$ spots PBMC). The 26 asymptomatic control subjects had few detectable TIV-specific ASCs (2 ± 5 spots/ 10^6 PBMC) after subtracting background spots from BSA coated wells. Thus a positive response was defined as >22 spots/million PBMC. Mean circulating total-IgG ASC number was 219 ± 209 spots/ 10^6 PBMC in this group. Concordantly, from the 6 vaccinated subjects, total-IgG ASC frequencies also peaked between days 5-8, and ranged from 1094 to 6033 spots/ 10^6 spots PBMC (mean $\pm$ SD, 4091 ± 2019 spots/ 10^6 PBMC) which was 5-30 fold above baseline values. TIV-specific ASCs returned to baseline by 15 days after immunization and all were negative on day 28. There was a strong correlation between the magnitude and timing of total-IgG and TIV-specific ASC numbers ($r=0.934$, $p<0.0001$).

Similar to the TIV-ASC results, we readily detected ASC responses to the individual H1 and H3 components of the TIV vaccine (figures 2 and 3). Not unexpectedly, the TIV-ASC responses, which include responses to the hemagglutinins of H1, H3 and B influenza, had greater frequencies than H1 and H3 measured separately. For both H1 and H3 the initial ASC response and the serum HAI responses occurred simultaneously and with similar magnitude. On average, peak TIV-, H1-, and H3-specific IgG ASCs constituted $63 \pm 21\%$, $26 \pm 10\%$, $22 \pm 17\%$, respectively, of the total-IgG ASCs number. Although variable for each of the subjects, the peak ASC frequency for each of the antigens always occurred 1-5 days (mean 2.3 ± 1.9 days for H1) prior to the maximum HAI antibody level. Only one subject failed to demonstrate an increase in HAI titer to H1 and H3 hemagglutinins following vaccination (subject A). This subject had the lowest H1-specific ASC response (maximum of 114 spots/million PBMCs) and also the lowest TIV ASC response. Similar to the TIV ASC kinetics, the peak H1-specific ASC frequencies ranged from day 5 to 8, with a mean peak frequency of 626 ± 520 spots/ 10^6 PBMC. Maximum H1 HAI titers were found between days 8-14 and were maintained through day 28. Three subjects had a small decline in H1 HAI at 6 months. The H3 ASC and HAI response pattern was similar to the H1 HAI and ASC responses with the maximum HAI titers also occurring approximately 1-5 days (mean 2.2 ± 1.9 days) after the peak ASC responses (mean of peak frequencies, 592 ± 463 spots/ 10^6 PBMC) (figure 3). Again, H3-specific ASC responses were not detected on day 28.

There was a strong correlation between H1-specific ASC responses and H1 HAI titer increases (Pearson coefficient $r=0.91$, $p=0.006$). There was a similar strong correlation between the area under the curve measurements for H1 ASC frequencies and fold-rise in

HAI titers (Pearson coefficient $r=0.96$, $p=0.0003$). In contrast, there was a relatively poor correlation between fold rise in H3 HAI and H3-ASC frequency ($r=0.2$, $p=0.5$). This may have been due to the mismatch between the H3 component in the TIV vaccine and the HAI measurements (A/Wyoming/H3) and the H3 used for the ASC assay (A/Wisconsin/H3). Even though the hemagglutinins to the matched H3 (Wisconsin) subtype and B influenza type eventually were obtainable; unfortunately, cells were not available from this study. Although there was a correlation between H1-ASC frequency and fold rise in H1-HAI, the number of subjects was too few to accurately determine a response threshold for ASCs that would correlate with a seroresponse after immunization.

The subject who received a tetanus vaccine had no detectable TIV-specific ASCs on days 4-10, 14, and 28, thus demonstrating the antigen specificity of the ASC assay. Peak tetanus-specific IgG frequencies were 528 ± 76 spots/ 10^6 PBMC on day 7 and were absent prior to and on day 28 after vaccination. Total-IgG ASC frequency prior to tetanus vaccination was 254 ± 77 spots/ 10^6 PBMC, reached 541 ± 58 spots/ 10^6 PBMC at the peak, and returned to 125 ± 22 spots/ 10^6 PBMC by day 28.

In addition to the antigen-specific Elispot assays, we measured cell surface markers to identify circulating ASCs or plasmablasts after vaccination by performing daily flow cytometry on matched blood samples in 4 of the subjects (C, D, E, and F). Plasmablasts were identified as cells with the following phenotype, $CD19^+IgD^-CD27^{hi}CD38^{hi}$. Figure 4A illustrates the increase in $CD38^{hi}CD27^{hi}$ plasmablasts, a subset of the $CD19^+IgD^-$ cells, from 5.5% to 17.4% on day 6 in subject D. In this subject, kinetics of the total plasmablasts identified by flow cytometry was similar to TIV-specific ASC and total IgG ASC frequencies demonstrated by Elispot assays (figure 4B). Similar results, in which the increase in $CD38^{hi}CD27^{hi}$ plasmablasts paralleled the rise in antigen specific ASC measured by Elispot was noted for each of the four subjects analyzed.

4. DISCUSSION

Following vaccination a rapid expansion of antigen-specific ASC occurs followed closely by a concordant rise in specific antibody levels to the immunogen. For influenza vaccination, it is known that influenza-specific ASCs can be detected in the blood several days after immunization; however, the precise timing of this increase, its variability between individuals and the relationship to the kinetics of antibody production has not been demonstrated. In this study, we demonstrate for the first time that after influenza vaccination, antigen-specific ASC can be detected as early as day 4-5, rapidly reaches a peak between days 5-8, followed by an equally rapid fall such that nearly all are negative by day 14. We found that the peak antigen-specific ASC frequency in the circulation correlates well with the fold-rise in serum HAI antibody levels after vaccination, when a well-matched antigen is used in all assays. Furthermore, there is a tight correlation between the AUC of antigen-specific ASCs with the fold-rise in serum antibody levels.

This tight correlation of ASC number and HAI response to H1 after vaccination had not been demonstrated in previous studies. Samples from the other studies were collected at days 7-10 (mean of day 9) and may have missed the peak ASC responses in the majority of subjects [9]. In contrast, we did not find a high correlation between the ASC frequencies, measured as either peak or AUC, with the fold-rise in HAI titer for H3 influenza A. This discrepancy may be due to the H3 mismatch between the H3 in the vaccine and the HAI assay, and that used for the ASC assay. This was somewhat surprising since there is 97% amino acid homology between the H3 of A/Wisconsin and A/Wyoming, but it may be a reflection of very high specificity of ASC assays that can detect divergent epitopes with high sensitivity. Future studies should explore this possibility.

For each of the seroresponsive subjects (B-F) the peak ASC number was noted 1-5 days prior to the peak HAI antibody titer. Although this would provide only a marginal time advantage in assessing vaccine responsiveness over a uniformly collected day 12-14 serum specimen, the use of an ASC assay requires only a single blood draw. Despite the overall correlation between peak ASC frequency and fold-rise in HAI titer, the limited number of subjects did not allow determination of a threshold that could be used to predict either a 4-fold or greater seroresponse, or a seroprotective titer of 1:40. However, the data does suggest that a frequency greater than ~ 350 ASC/ 10^6 PBMC is needed since the one non-responder had peak ASC frequencies of ~ 100 , while all of the seroresponders had peak frequencies above $350/10^6$ PBMC. Future studies with larger numbers of subjects will be required to establish such a surrogate of seroresponsiveness or seroprotection.

One factor that is difficult to control for in this type of study is the effect of previous influenza vaccination or infection, and it is possible that such exposures could influence the magnitude of the influenza-specific ASC frequencies. Subject A had the lowest frequency of influenza-specific ASCs and failed to sero-respond. Interestingly, this subjects reported annual influenza vaccination in each of the previous five years. Subjects C and D had a history of one previous vaccination and also recalled an influenza-like illness in the past several years. Subjects B, E and F had no history of previous vaccinations and could not recall recent influenza-like illnesses.

Although we report only a single subject who was immunized with a non-influenza antigen, we have repeatedly observed a high degree of antigen-specificity of the ASC assay following infection with several respiratory viruses and other vaccines (unpublished observations). Most other reports describe very little non-antigen-exposed antibody production after vaccination [13-15]. However, Bernasconi et al reported one subject after tetanus vaccination who may have had non-antigen-specific ASC responses [16]. Since ASC Elispot assays are highly sensitive, they are ideal for detection of very low ASC frequencies, but their study demonstrates non-antigen-exposed responses of 0.01-1 cell/ 10^6 PBMC which are near the limits of detection, and at the background values, of our ASC Elispots assays (0.01-1 cell/million PBMC). Thus, the question of low level bystander activation of non-antigen exposed B cells remains open.[16].

Circulating antigen-specific ASCs can function as a window to understanding the long-lived antibody responses since these cells are easily assessable and amenable to repeat sampling. Thus, understanding the kinetics of the blood ASCs which can constitute up to 90% of the antigen-specific responses is important as an ideal early biomarker of vaccine responsiveness. Furthermore, identifying heterogeneity of these antigen-specific ASCs as cellular biomarkers of humoral vaccine immunity is likely to predict short and long-lived antibody responses. We found that the kinetics of ASC identified by surface markers $CD19^+IgD^-CD27^{hi}CD38^{hi}$ are similar to the antigen-specific responses and thus could potentially be used as another surrogate marker of vaccine response. It is likely that specific subset(s) of these cells is associated with long-lived potential. Thus, an understanding of the complex heterogeneity of blood ASCs after antigen exposure may be useful in forecasting the duration and location of humoral responses. Furthermore, the ability to identify ASCs using non-antigen-specific flow cytometry markers could be used to characterize these cells for clonality, survival potential, apoptosis, chemokine expression, and cytokine production. Lastly, the utility of the antigen-specific ASC has proven invaluable for the generation of fully humanized antigen-specific monoclonal antibodies on a single cell level [17,18]. Therefore, knowledge of their kinetics in the blood after vaccination can maximize the yield of these rare cells.

In conclusion, we demonstrate that the ASCs found in the blood after recent vaccination are specific to the antigen of exposure and the peak ASC frequency correlates with the rise in antibody titer. As an early biomarker of vaccine response, it may gain only 1-5 days from a rise of serum antibody titers. However, only a single time point is required and may be useful to rapidly identify non-responsive individuals.

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Abbreviations

ASC	Antibody secreting cell
PBMC	peripheral blood mononuclear cells
TIV	trivalent inactivated influenza vaccine

REFERENCES

- [1]. Mei HE, Yoshida T, Sime W, Hiepe F, Thiele K, Manz RA, et al. Blood-borne human plasma cells in steady-state are derived from mucosal immune responses. *Blood*. Nov 5.2008
- [2]. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity*. Mar; 1998 8(3):363–72. [PubMed: 9529153]
- [3]. Dorner T, Radbruch A. Antibodies and B cell memory in viral immunity. *Immunity*. Sep; 2007 27(3):384–92. [PubMed: 17892847]
- [4]. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat Rev Immunol*. Oct; 2006 6(10):741–50. [PubMed: 16977339]
- [5]. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. *Scand J Immunol*. Jan; 2004 59(1):1–15. [PubMed: 14723616]
- [6]. Cox NJ, Subbarao K. Influenza. *Lancet*. Oct 9; 1999 354(9186):1277–82. [PubMed: 10520648]
- [7]. Nichol KL, Treanor JJ. Vaccines for seasonal and pandemic influenza. *J Infect Dis*. Nov 1; 2006 194(Suppl 2):S111–8. [PubMed: 17163383]
- [8]. Sasaki S, He XS, Holmes TH, Dekker CL, Kemble GW, Arvin AM, et al. Influence of prior influenza vaccination on antibody and B-cell responses. *PLoS ONE*. 2008; 3(8):e2975. [PubMed: 18714352]
- [9]. Sasaki S, Jaimes MC, Holmes TH, Dekker CL, Mahmood K, Kemble GW, et al. Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. *J Virol*. Jan; 2007 81(1): 215–28. [PubMed: 17050593]
- [10]. Noble GR, Kaye HS, Yarbrough WB, Fiedler BK, Reed CJ, Felker MB, et al. Measurement of hemagglutination-inhibiting antibody to influenza virus in the 1976 influenza vaccine program: methods and test reproducibility. *J Infect Dis*. Dec; 1977 136(Suppl):S429–34. [PubMed: 342617]
- [11]. Kyu SY, Kobie J, Yang H, Zand MS, Topham DJ, Quataert SA, et al. Frequencies of human influenza-specific antibody secreting cells or plasmablasts post vaccination from fresh and frozen peripheral blood mononuclear cells. *J Immunol Methods*. Jan 1; 2009 340(1):42–7. [PubMed: 18996127]

- [12]. Kyu S-Y, Kobie J, Zand MS, Topham DJ, Quataert SA, Sanz I, et al. Frequencies of Human Influenza-specific Antibody Secreting Cells or Plasmablasts post Vaccination from Fresh and Frozen PBMC. *Journal of Immunological Methods*. 2008 In Press.
- [13]. Di Genova G, Roddick J, McNicholl F, Stevenson FK. Vaccination of human subjects expands both specific and bystander memory T cells but antibody production remains vaccine specific. *Blood*. Apr 1; 2006 107(7):2806–13. [PubMed: 16339400]
- [14]. Nanan R, Heinrich D, Frosch M, Kreth HW. Acute and long-term effects of booster immunisation on frequencies of antigen-specific memory B-lymphocytes. *Vaccine*. Nov 12; 2001 20(3-4):498–504. [PubMed: 11672915]
- [15]. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*. Nov 8; 2007 357(19):1903–15. [PubMed: 17989383]
- [16]. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science*. Dec 13; 2002 298(5601):2199–202. [PubMed: 12481138]
- [17]. Wrammert J, Smith K, Miller J, Langley WA, Kokko K, Larsen C, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature*. 2008; 453(7195):667–71. [PubMed: 18449194]
- [18]. Jin A, Ozawa T, Tajiri K, Obata T, Kondo S, Kinoshita K, et al. A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-secreting cells from human peripheral blood. *Nat Med*. Aug 16.2009

Figure 1.

Kinetics of ASCs in the blood after immunization with trivalent influenza vaccine (TIV) in 6 subjects. ASC are identified that respond to all three hemagglutinin components (H1, H3 and B) of TIV. Total IgG ASCs are indicated by thin black triangles, BSA-specific ASC by open triangles, and TIV-specific ASC by bold black circles.

Figure 2.
Kinetics of H1-specific ASC in the blood after TIV immunization in 6 subjects (solid triangles). Kinetics of H1-specific HAI antibody is shown by open triangles.

Figure 3.
Kinetics of H3-specific ASC in the blood after TIV immunization in 6 subjects (solid circles). Kinetics of H3-specific HAI antibody is shown by open circles.

Figure 4.

Comparison of circulating ASC or plasmablasts by flow cytometry and total IgG Elispot assays. (A) Plasmablasts identified by CD19⁺IgD⁻CD27^{hi}CD38^{hi} on day 0 and 6 for subject D. (B) Kinetics of ASC identified by flow cytometry and both total IgG and TIV-specific Elispots assays in subject D.