

REVIEW ARTICLE

Downfall of the current antibody correlates of influenza vaccine response in yearly vaccinated subjects: Toward qualitative rather than quantitative assays

Alberto Cagigi¹, Nicola Cotugno^{1,2}, Stefano Rinaldi², Veronica Santilli¹, Paolo Rossi^{1,2} & Paolo Palma¹

¹University Department of Pediatrics, DPUO, Unit of Immune and Infectious Diseases, Bambino Gesù Children's Hospital, Rome, Italy; ²Chair of Pediatrics, Department of System Medicine, University of Rome Tor Vergata, Rome, Italy

To cite this article: Cagigi A, Cotugno N, Rinaldi S, Santilli V, Rossi P, Palma P. Downfall of the current antibody correlates of influenza vaccine response in yearly vaccinated subjects: toward qualitative rather than quantitative assays. *Pediatr Allergy Immunol* 2015; **00**.

Keywords

repeated immunization; long-lasting immunologic memory; antibody vs. memory B-cell responses

Correspondence

Alberto Cagigi, Biodefense Research Section, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 40 Convent Drive, Bldg. 40/2609B, Bethesda, Maryland, USA

Tel.: +1 301 919 7923

E-mail: alberto.cagigi@gmail.com

Accepted for publication 2 September 2015

DOI:10.1111/pai.12483

Abstract

Response to seasonal influenza vaccination is currently evaluated by antibody correlates that estimate vaccine seroconversion as well as immune protection. These correlates rely on the general dogmas surrounding seasonal influenza vaccination; that is, that vaccine-induced antibodies would exclusively generate immunity to influenza vaccine strains and that protective immunity would wane before the next season. Here, we summarize recently reported data on immunity to seasonal influenza in healthy individuals and rediscuss results on yearly vaccinated pediatric immunocompromised patients that together highlight the need for revision of the current correlates of vaccine response to shift from quantitative to qualitative measurements.

Influenza viruses are highly variable; therefore, new vaccines must be developed every year. Usually, trivalent formulations, that is, containing three inactivated influenza virus strains, are developed by different pharmaceutical companies and made commercially available in the late summer–early fall, before seasonal outbreaks occur. The vaccine strains are chosen according to predictions by the World Health Organization (WHO) based on strains that will most likely circulate during the next season. Usually, seasonal vaccines contain two influenza type A virus strain subtypes (H1N1 and H3N2) and one influenza type B virus strain. The committee for proprietary medicinal products (CPMP) has established that the response to each vaccination should be determined by estimated levels of antibodies measured by hemagglutination inhibition (HI) assays, which reflect protection. These estimated levels became universally accepted and turned into standards for evaluating and licensing new influenza vaccines in Europe and in the rest of the world ([http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/20](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf)

09/09/WC500003945.pdf). According to these standard correlates, vaccine seroconversion occurs with an at least four-fold antibody increase from baseline, whereas absolute HI titers ≥ 40 are considered to mirror protective immunity (1). Due to increased risk of complications in case of influenza infection, yearly immunization against seasonal influenza is recommended for young children, elderly patients with chronic diseases and immunocompromised individuals (2). On the contrary, a large portion of young/middle-aged healthy individuals does not generally vaccinate against seasonal flu.

Factors that may generally account for incorrect interpretation of vaccine efficacy with the current antibody correlates

The general dogmas surrounding seasonal influenza vaccination dictate that vaccine-induced antibodies would exclusively generate immunity to the same virus strains when circulating during the outbreak season. As a consequence, exposure to

strains not included in the vaccine formulation would cause disease. In addition, vaccine-induced protective immunity is believed to wane rapidly; therefore, revaccination with the same vaccine strains would be necessary in case these are predicted to be circulating again during the following season (3). However, among the recently published research on influenza, contrasting data on these dogmas jeopardize the rationale behind the current antibody correlates of vaccine-mediated immune protection. For example, Hiroi et al. (4) demonstrated cross-reactivity of antibodies generated with vaccination against H3N2 (A/Victoria/210/2009) to five different strains of H3N2 viruses circulating between 2010 and 2012. On the other hand, He et al. (5) studied the specificity of plasmablasts generated either by natural infection or by vaccination with A(H1N1)pdm09 and observed that only plasmablast-derived antibodies arisen because of natural infection targeted conserved epitopes and thus had potential for cross-reactivity between strains. However, He et al. (5) also observed that among the vaccinees, neutralizing antibodies against A(H1N1)pdm09 were present in serum prior to vaccination. This may suggest that either these individuals may have residual immunity from previous vaccination with A(H1N1)pdm09 or that exposure to circulating influenza virus strains other than the vaccine strain might have in fact generated cross-reactive antibodies. Interestingly, Jackson et al. (6) performed sequence analyses after pandemic (A) H1N1pdm09 vaccination and observed the same type of antibody heavy chain gene family rearrangement in different people. In view of this, one may speculate that exposure to circulating influenza viruses could similarly generate a restricted pool of antigen-specific B cells. In these hypothetical settings, the possibility that unmutated common ancestor B cells would give rise to a spectrum of slightly divergent B-cell clones by somatic hypermutation is likely. This might increase antibody cross-reactivity to drift variant strains. On the other hand, Andrews et al. (7) recently reported that B-cell response and antibody levels are consistently tuned down in case of preexisting immunity to a given influenza strain upon re-immunization in contrast to response against a new strain. This may particularly impact on the evaluation of vaccines containing recurrent strains over the years. Altogether, these data and observations not only arise concerns on the truthfulness of the general dogmas surrounding seasonal influenza vaccination but, more importantly, arise concerns on the ease of misinterpretation of the standard antibody correlates to the point that the CPMP recently withdrew their previous notes on influenza vaccination (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500161022.pdf).

Response to seasonal influenza vaccination in immunocompromised individuals

Advances in the treatment of a number of pediatric infectious diseases have, during the last decade, led to partial or full restoration of immune functions in children and young individuals. Among all immune diseases, a clear example can be found in HIV-1 infection. Early antiretroviral therapy

(ART) is associated with a better survival rate in both adults and children (8, 9). Furthermore, we have previously shown that vertically infected children are able to preserve the normal development of B-cell functions if ART is initiated during the first year of life (10, 11). Likewise in adults, higher degrees of immune reconstitution are most likely to be obtained if ART is initiated during the early stages of primary infection (12). Following these discoveries, ART is now applied to HIV-1-infected patients, both children and adults, as early as possible and results of early ART can now be appreciated also in the context of influenza vaccination. For example, in a randomized clinical trial, McKittrick et al. (13) compared the immunogenicity of a high- and a standard-dose influenza vaccine in HIV-1-positive subjects side by side. As it would be expected for healthy subjects, also HIV-1-infected individuals reached higher levels of influenza seroprotection if vaccinated with the high-dose trivalent vaccine than with the standard dose. In another longitudinal study, Berger et al. reconstructed influenza A and B vaccine response profiles of HIV-1-infected individual and healthy controls taking into account different parameters including preexisting immunity (antibody and T-cell responses) and vaccine preparation. Results pointed out that similar fluctuations of the above parameters occurred in both populations (14). Nevertheless, the majority of HIV-1-infected and other immunocompromised patients, according to recommendations, keep undergoing seasonal influenza vaccination yearly. This recently lead to discussions on whether the current standard antibody correlates would generally be appropriate for patients considered as immune deficient, but potentially stable, who perform yearly vaccination and at the same time for healthy individuals who are not subjected to routine vaccination (http://www.who.int/immunization/research/meetings_workshops/en/). These correlates have in fact been validated in healthy subjects, most likely, after single vaccination.

Downfall of the current antibody correlates: A(H1N1)pdm09 as a model of antigenic discontinuity

Interestingly, it has recently been hypothesized that the generation of long-lasting immunologic memory may depend on antigenic discontinuity, that is, when an antigen challenges the immune system frequently and intermittently (15). This theory could be applicable to the administration of seasonal influenza vaccines because the same A(H1N1)pdm09 strain is being included in seasonal vaccine formulations since 2009. According to this theory, the expected magnitude and durability of immune responses to A(H1N1)pdm09 should differ between immunocompromised patients (with normal lymphocyte numbers and function) who are vaccinated yearly, and healthy individuals who do not perform routine vaccination. We recently evaluated both antibody and memory B-cell strain-specific responses to the trivalent 2012–2013 seasonal influenza vaccine in two different cohorts of immunocompromised pediatric patients, HIV-1 vertically infected and kidney transplant, as compared to response in age-matched healthy subjects (16, 17). HIV-1-infected and kidney transplant

patients can be considered as different models of secondary immune deficiency for the following reasons: (i) Both may experience chronic immune activation due to the persistence of a foreign antigen, that is, HIV-1 and the transplanted organ, and (ii) both are considered as immune deficient because of direct depletion of CD4⁺ T cells due to HIV-1 infection and because of the application of immunosuppressive therapies after transplantation. Interestingly, in our studies, according to the standard correlates based on the HI assay, a high percentage of HIV-1-infected and kidney transplant patients presented with protective immunity to A(H1N1)pdm09 prior to administration of the 2012–2013 seasonal vaccine and did not seroconvert after vaccination (16, 17). Whereas seroconversion rates inversely related with the number of times that the patients had previously been vaccinated with formulations containing A(H1N1)pdm09, the percentage of seroprotected individuals prior to vaccination followed an opposite trend with the highest number of seroprotected subjects also being the most frequently vaccinated. On the other hand, A(H1N1)pdm09-specific memory B cells increased slightly in all individuals. Altogether, this suggests that upon repeated challenge, A(H1N1)pdm09-specific memory B cells generated during previous vaccinations may undergo limited divisions, resulting in lower antibody production (Fig. 1). This has also been reported for plasmablasts in healthy individuals by He et al. (5) who observed that if immunologic priming was due to vaccination and not to natural infection, reduced plasmablast responses to a subsequent immunization would occur and, as previously discussed, by Andrews et al. (7). Although data are still missing in humans, new evidence reported by McHeyzer-Williams et al. (18) in mice describe the diversity of the antigen-specific B-cell repertoire induced by vaccine booster over time. These data suggest that the selection of highly specific memory B-cell clones through somatic hypermutation after antigenic boost should be monitored with other parameters assessing the quality rather than the quantity of antibody

ies produced, especially in yearly vaccinated patients. Altogether, these studies clearly indicate that with yearly administration of influenza vaccines containing the same A(H1N1)pdm09 strain, the current standard correlates do fail to distinguish between vaccine responders and non-responders (5, 7, 16, 17) as well as possibly between protected vs. non-protected individuals. In yearly exposed healthy individuals indeed, a lack of association between pre-season HI titers and protection from natural infection by A(H1N1) virus strains was recently reported (19).

Alternative more advanced tools to evaluate seasonal influenza vaccine efficacy in yearly vaccinated patients

The current antibody correlates of influenza vaccine response and immune protection must be revised and the introduction of new parameters evaluated, particularly for immunocompromised patients yearly vaccinated with seasonal influenza. One easy alternative could, for example, be testing the measurement of antibody titers to neuraminidase as Monto et al. (20) observed that upon vaccination, despite these arise in fewer individuals as compared to antibodies against HA, they do correlate with clinical protection. Similarly, results from microneutralization assays have also recently been shown to correlate with immune protection against seasonal H1N1 and H3N2 in children and could therefore be a candidate for further evaluation (21). Another feasible possibility could be measuring the modulation of strain-specific B cells and their numbers before vaccination, in addition to antibody titers, as we have shown in our studies (16, 17). This will at least add information for evaluating the response to new seasonal vaccines as well as possibly for evaluating immune protection prior to undergoing vaccination with the same influenza strains.

However, in order to move forward in the search for novel and more comprehensive immune correlates, more advanced

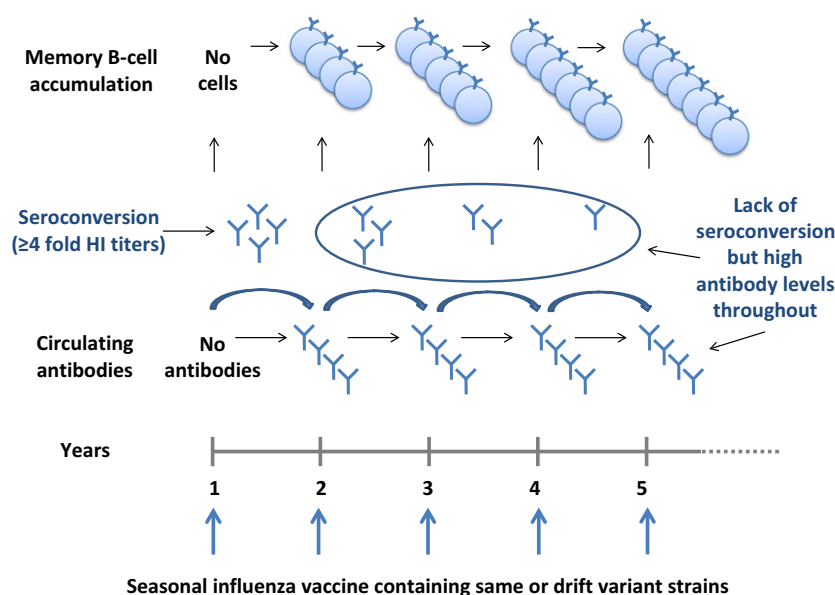


Figure 1 The A(H1N1)pdm09 model. Upon yearly challenge, A(H1N1)pdm09-specific memory B cells may undergo fewer divisions resulting in lower antibody production. As a consequence, only a moderate increase in these cells would occur upon encounter with the same vaccine antigen.

tools to study the ontogeny of antigen-specific immunoglobulins and of B-cell responses upon influenza vaccination should be used. Other hallmarks of vaccine efficacy might, for example, be identified through the characterization of the antigen-specific antibody repertoire by sequencing the immunoglobulin variable region of B cells sorted with specific probes (22). The diversity of the antibody repertoire reflects the individual's ability to effectively select B cells able to produce high-affinity antibodies through somatic hypermutation upon prime/boost in germinal centers. Such analysis may not only reveal new qualitative correlates of vaccine response and/or vaccine-mediated immune protection in yearly vaccinated patients but, importantly, might be pivotal for evaluating the

above parameters in patients suffering from diseases affecting secondary lymphoid organs.

In addition, immunoglobulin subclasses, glycosylation (23), and the ability of antibodies to engage different types of Fc receptors (24) should be investigated as these characteristics may impact antibody effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent complement deposition (ADCD) (<http://www.croiconference.org/sessions/potentiating-protective-antibody-activity-systems-serology-approach>). In line with this, functional assays to investigate the ability of vaccine-derived antibodies to mediate such effector functions should also be performed (Fig. 2).

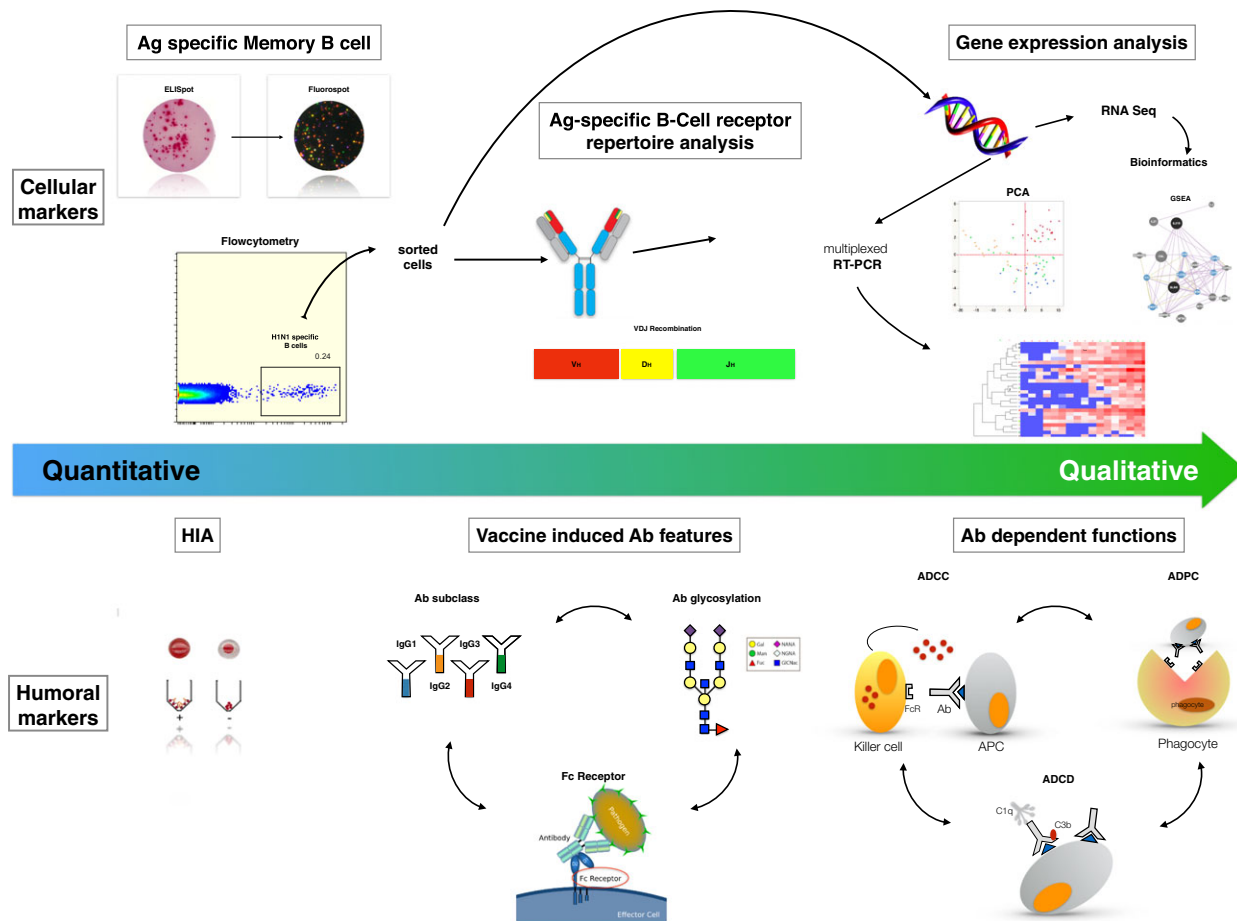


Figure 2 Toward qualitative assays of vaccine-induced antibodies and memory B cells. The upper part of the figure shows quantitative assays to measure antigen-specific B cells, that is, enzyme-linked immunosorbent spot (ELISpot) and fluorospot. This latter has more recently supplemented the ELISpot technique with the possibility of immune phenotyping by multiple secretory marker staining. Cell staining and sorting with antigen-specific probes by flow cytometry, in addition to allowing quantitative analyses, represents the first step to move toward assessment of quality, for example, by sequencing and characterizing the B-cell receptor repertoire and by studying gene expression through RNA-Seq and multiplexed RT-PCR (Fluidigm, Biomark). The lower part of the figure highlights how novel correlates of vaccine response could perhaps reflect on the different antibody features and effector functions. VH, variable domain of the heavy chain; DH, diversity domain of the heavy chain; JH, joining domain of the heavy chain; PCA, principal component analysis; GSEA, gene set enrichment analysis; HC, hierarchical clustering; HIA, hemagglutination inhibition assay; ADCC, antibody-dependent cytotoxicity; ADPC, antibody-dependent phagocytosis; ADCD, antibody-dependent complement deposition.

The molecular mechanisms behind protective B-cell responses to influenza virus infection, immunization and antigen recall, are still unknown. Recent evidence in the field of vaccinomics pointed out to specific genes being modulated *in vivo* after vaccination (25, 26). In this context, mathematical modeling and bioinformatics could be applied to correlate transcriptome analysis to assays evaluating vaccine efficacy (27, 28). Baseline expression of different genes has also been studied to identify predictive biomarkers of immune response (29–31). In this respect, antigen-specific B-cell quality, in terms of expression of different genes involved in immune activation, crucially contributes to the production of high-affinity antibodies (18, 32). However, these data should probably be considered as indicative as they have been produced in total peripheral blood mononuclear cells (PBMC) from healthy individuals and not in sorted B cells. In the context of immunocompromised patients, alterations of the immune phenotype might directly relate to disease. In this respect, we have shown that immunocompromised individuals might present with increased frequencies of senescent-like and activated B cells which might negatively impact response to influenza and other vaccinations (33, 34). Whether these cells produce influenza-specific antibodies and what their quality is currently remain unknown. Altogether, this highlights the need to analyze transcriptome differences in sorted B-cell subtypes from immunocompromised individuals (Cotugno N. J. of Virus Eradication. In press). High-throughput analyses (RNA-Seq or multiplexed RT-PCR) on specific B/T-cell subsets or antigen-specific cells may therefore uncover predictive models of vaccine response in chronically infected patients routinely vaccinated with the same influenza strain, thus depicting the ability of such patients to respond to vaccination through the description of host-related immune characteristics (Fig. 2).

Conclusions

Recent evidence, although on occasion contrasting, strongly points to questioning the dogmas surrounding the mechanisms

of action triggered by influenza vaccination. This automatically translates into questioning the interpretation of vaccine efficacy and immune protection to the different vaccine strains which is now based on antibody correlates. Recommendations and immunization strategy for potentially high-risk populations are based on these correlates, and this has so far led to multiple and repeated vaccination of these individuals. Although this might not be particularly important for the healthy population, a different scenario might present for high-risk populations, especially for subjects having their immune system constantly under stress because of chronic immune activation. In this respect, we would like to draw the attention to another study in which we have shown that possibly unnecessary challenge of the immune system with A(H1N1)pdm09 increased the concentration of unspecific antibodies and thus the probability for autoimmune phenomena in yearly vaccinated HIV-1 vertically infected and kidney transplant pediatric patients (35). This represents an example that points to the need for personalized vaccination strategies for individuals belonging to the large and heterologous group of patients classified as immunocompromised, at least in the case of repeated immunization with the same influenza strains.

Acknowledgments

We wish to thank Isabella Donatelli, Angela Di Martino, and Maria Rita Castrucci from the WHO National Influenza Centre at the Istituto Superiore di Sanità, Rome, Italy, for performing and analyzing the HI assays for our hereby discussed studies. We also wish to thank Luca Dello Strologo and his staff and Paolo Rossi's staff at the Bambino Gesù Children's Hospital, Rome, Italy, for taking care of the patients enrolled in our hereby discussed studies. Finally, we would like to thank Jennifer Faudella and Raffaella Addario for their precious administrative work.

References

1. Cagigi A, Cotugno N, Giaquinto C, et al. Immune reconstitution and vaccination outcome in HIV-1 infected children: present knowledge and future directions. *Hum Vaccin Immunother* 2012; **8**: 1784–94.
2. Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices–United States, 2013–2014. *MMWR Recomm Rep* 2013; **62**: 1–43.
3. Chiu C, Ellebedy AH, Wrammert J, Ahmed R. B cell responses to influenza infection and vaccination. *Curr Top Microbiol Immunol* 2015; **386**: 381–98.
4. Hiroi S, Morikawa S, Nakata K, et al. Trivalent influenza vaccine-induced antibody response to circulating influenza A (H3N2) viruses in 2010/11 and 2011/12 seasons. *Hum Vaccin Immunother* 2015; **11**: 386–90.
5. He XS, Holmes TH, Sanyal M, et al. Distinct patterns of B-cell activation and priming by natural influenza virus infection versus inactivated influenza vaccination. *J Infect Dis* 2015; **211**: 1051–9.
6. Jackson KJ, Liu Y, Roskin KM, et al. Human responses to influenza vaccination show seroconversion signatures and convergent antibody rearrangements. *Cell Host Microbe* 2014; **16**: 105–14.
7. Andrews SF, Kaur K, Pauli NT, Huang M, Huang Y, Wilson PC. High preexisting serological antibody levels correlate with diversification of the influenza vaccine response. *J Virol* 2015; **89**: 3308–17.
8. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med* 2008; **359**: 2233–44.
9. Reniers G, Slaymaker E, Nakiyingi-Miiró J, et al. Mortality trends in the era of antiretroviral therapy: evidence from the Network for Analysing Longitudinal Population based HIV/AIDS data on Africa (ALPHA). *AIDS* 2014; **28** (Suppl. 4): S533–42.
10. Pensiero S, Cagigi A, Palma P, et al. Timing of HAART defines the integrity of memory B cells and the longevity of humoral responses in HIV-1 vertically-infected children. *Proc Natl Acad Sci USA* 2009; **106**: 7939–44.
11. Cagigi A, Rinaldi S, Cotugno N, et al. Early highly-active antiretroviral therapy enhances B-cell longevity: a 5 year follow-up. *Pediatr Infect Dis J* 2014; **33**: 126–31.

12. Titanji K, Chiodi F, Bellocco R, et al. Primary HIV-1 infection sets the stage for important B lymphocyte dysfunctions. *AIDS* 2005; **19**: 1947–55.
13. McKittrick N, Frank I, Jacobson JM, et al. Improved immunogenicity with high-dose seasonal influenza vaccine in HIV-infected persons: a single-center, parallel, randomized trial. *Ann Intern Med* 2013; **158**: 19–26.
14. Berger CT, Greiff V, Mehling M, et al. Influenza vaccine response profiles are affected by vaccine preparation and preexisting immunity, but not HIV infection. *Hum Vaccin Immunother* 2015; **11**: 391–6.
15. Pradeu T, Jaeger S, Vivier E. The speed of change: towards a discontinuity theory of immunity? *Nat Rev Immunol* 2013; **13**: 764–9.
16. Rinaldi S, Zangari P, Cotugno N, et al. Antibody but not memory B-cell responses are tuned-down in vertically HIV-1 infected children and young individuals being vaccinated yearly against influenza. *Vaccine* 2014; **32**: 657–63.
17. Rinaldi S, Cagigi A, Santilli V, et al. B-sides serologic markers of immunogenicity in kidney transplanted patients: report from 2012–2013 flu vaccination experience. *Transplantation* 2014; **98**: 259–66.
18. McHeyzer-Williams LJ, Milpied PJ, Okitsu SL, McHeyzer-Williams MG. Class-switched memory B cells remodel BCRs within secondary germinal centers. *Nat Immunol* 2015; **16**: 296–305.
19. Fox A, Mai LQ, Thanh LT, et al. Hemagglutination inhibiting antibodies and protection against seasonal and pandemic influenza infection. *J Infect* 2014; **70**: 187–96.
20. Monto AS, Petrie JG, Cross RT, et al. Antibody to influenza virus neuraminidase: an independent correlate of protection. *J Infect Dis* 2015; **212**: 1191–9.
21. Verschoor CP, Singh P, Russell ML, et al. Microneutralization assay titres correlate with protection against Seasonal influenza H1N1 and H3N2 in children. *PLoS One* 2015; **10**: e0131531.
22. Boyd SD, Jackson KJ. Predicting vaccine responsiveness. *Cell Host Microbe* 2015; **17**: 301–7.
23. Ackerman ME, Crispin M, Yu X, et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. *J Clin Invest* 2013; **123**: 2183–92.
24. DiLillo DJ, Tan GS, Palese P, Ravetch JV. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus *in vivo*. *Nat Med* 2014; **20**: 143–51.
25. Nakaya HI, Li S, Pulendran B. Systems vaccinology: learning to compute the behavior of vaccine induced immunity. *Wiley Interdiscip Rev Syst Biol Med* 2012; **4**: 193–205.
26. Pulendran B. Systems vaccinology: probing humanity's diverse immune systems with vaccines. *Proc Natl Acad Sci USA* 2014; **111**: 12300–6.
27. Poland GA, Kennedy RB, McKinney BA, et al. Vaccinomics, adversomics, and the immune response network theory: individualized vaccinology in the 21st century. *Semin Immunol* 2013; **25**: 89–103.
28. Hagan T, Nakaya HI, Subramaniam S, Pulendran B. Systems vaccinology: enabling rational vaccine design with systems biological approaches. *Vaccine* 2015; **33**: 5294–301.
29. Zou W, Lackner AA, Simon M, et al. Early cytokine and chemokine gene expression in lymph nodes of macaques infected with simian immunodeficiency virus is predictive of disease outcome and vaccine efficacy. *J Virol* 1997; **71**: 1227–36.
30. Maertzdorf J, Kaufmann SH, Weiner J 3rd. Molecular signatures for vaccine development. *Vaccine* 2015; **33**: 5256–61.
31. Tan Y, Tamayo P, Nakaya H, Pulendran B, Mesirov JP, Haining WN. Gene signatures related to B-cell proliferation predict influenza vaccine-induced antibody response. *Eur J Immunol* 2014; **44**: 285–95.
32. De Silva NS, Klein U. Dynamics of B cells in germinal centres. *Nat Rev* 2015; **15**: 137–48.
33. Cagigi A, Rinaldi S, Di MA, et al. Premature immune senescence during HIV-1 vertical infection relates with response to influenza vaccination. *J Allergy Clin Immunol* 2013; **133**: 592–4.
34. Cotugno N, Finocchi A, Cagigi A, et al. Defective B-cell proliferation and maintenance of long-term memory in patients with chronic granulomatous disease. *J Allergy Clin Immunol* 2015; **135**: 753–61 e2.
35. Cagigi A, Rinaldi S, Santilli V, et al. Premature ageing of the immune system relates to increased anti-lymphocyte antibodies (ALA) after an immunization in HIV-1-infected and kidney-transplanted patients. *Clin Exp Immunol* 2013; **174**: 274–80.