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One dose of an MF59-adjuvanted pandemic A/H1N1 vaccine recruits pre-existing immune memory and induces the rapid rise of neutralizing antibodies

Elisa Faenzi[a,1], Luisanna Zedda[a,1], Monia Bardelli[a], Fabiana Spensieri[a], Erica Borgogni[a], Gianfranco Volpini[a], Francesca Buricchi[a], Franco Laghi Pasini[c], Pier Leopoldo Capecchi[c], Fabio Montanaro[d], Riccardo Belli[b], Maria Lattanzi[b], Simona Piccirella[e], Emanuele Montomoli[e], Syed Sohail Ahmed[a], Rino Rappuoli[a], Giuseppe Del Giudice[a], Oretta Finco[a], Flora Castellino[a], Grazia Galli[a,*]

[a] Research Development, Novartis Vaccines and Diagnostics srl, Siena, Italy
[b] Clinical Development, Novartis Vaccines and Diagnostics srl, Siena, Italy
[c] Department of Clinical Medicine and Immunological Sciences, University of Siena, Siena, Italy
[d] OPERA, Genova, Italy
[e] Department of Physiopathology, Experimental Medicine and Public Health, University of Siena, Siena, Italy

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ABSTRACT

Protective antibody responses to a single dose of 2009 pandemic vaccines have been observed in the majority of healthy subjects aged more than 3 years. These findings suggest that immune memory lymphocytes primed by previous exposure to seasonal influenza antigens are recruited in the response to A/H1N1 pandemic vaccines and allow rapid seroconversion. However, a clear dissection of the immune memory components favoring a fast response to pandemic vaccination is still lacking. Here we report the results from a clinical study where antibody, CD4+ T cell, plasmablast and memory B cell responses to one dose of an MF59-adjuvanted A/H1N1 pandemic vaccine were analyzed in healthy adults. While confirming the rapid appearance of antibodies neutralizing the A/H1N1 pandemic virus, we show here that the response is dominated by IgG-switched antibodies already in the first week after vaccination. In addition, we found that vaccination induces the rapid expansion of pre-existing CD4+ T cells and IgG-memory B lymphocytes cross-reactive to seasonal and pandemic A/H1N1 antigens. These data shed light on the different components of the immune response to the 2009 H1N1 pandemic influenza vaccination and may have implications in the design of vaccination strategies against future influenza pandemics.

1. Introduction

When in 2009 a new A/H1N1 virus spread rapidly worldwide, the race to produce vaccines against the pandemic virus started immediately. Based on results available at that time, it was not possible to predict whether 2 doses of adjuvanted vaccines would have been needed, even in adults, to induce protective antibodies as observed in pre-pandemic studies with H5N1 vaccines [1–3]. Cross-protective antibodies to the 2009 A/H1N1 pandemic virus, as assessed by hemagglutinin inhibition (HI) or micro-neutralization (MN) assays, were found only in 30% of subjects aged 60 years or older, while they were barely detectable in subjects born after the 1950s [4]. These results challenged the hypothesis that pre-existing immune memory in the human population could substantially contribute to speed the antibody response to pandemic vaccines. Surprisingly, clinical studies with 2009 monovalent pandemic vaccines showed that more than 70% of subjects between 3 and 50 years of age responded rapidly to a single dose, acquiring in less than 3 weeks circulating HI and MN antibodies at levels considered protective for seasonal influenza vaccines [5–8]. These results suggested that immune memory cross-reactive to pandemic influenza antigens could be present in the population and also in young children who, eventually, had only been exposed to seasonal influenza virus circulating in the 3 years before the pandemic. Indeed, experiments performed in ferrets demonstrated that even a single dose of the 2008–2009 seasonal influenza vaccine, while not effective at inducing cross-reactive antibodies, was sufficient to prime immune memory responses cross-reactive to pandemic A/H1N1 antigens.

* Corresponding author at: Research Center, Novartis Vaccines and Diagnostics srl, 53100, Siena, Italy. Tel.: +39 0577 539007; fax: +39 0577 278600.
E-mail address: grazia.galli@novartis.com (G. Galli).
[1] Elisa Faenzi and Luisanna Zedda contributed equally to this work.

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Animals primed with MF-59-adjuvanted seasonal vaccines and boosted with one dose of MF59-adjuvanted pandemic vaccines had superior HI and MN antibody responses and increased resistance to pandemic influenza challenge [9]. More recent retrospective analyses of clinical data have shown that subjects who had been infected by, or vaccinated against, seasonal influenza strains circulating from 2007 to 2010 had elevated levels of HI and MN antibodies against the 2009 pandemic strain [10,11].

Results from *in silico* and *in vitro* studies have demonstrated that memory CD4+ T cells cross-reactive to pandemic H1N1 antigens circulated in the blood of adults not exposed to pandemic H1N1 antigens [12–14]. However, their actual recruitment in response to pandemic vaccination is still poorly defined. Even less clear is the potential contribution of pre-existing memory B cells. The substantial structural diversity in the HA1 domain found between the 2009 pandemic virus and seasonal H1N1 strains that circulated from 1950s provides a mechanistic explanation to the lack of HI cross-reactive antibodies in sera collected from young adults before 2009 [15,16]. Still it remains unclear whether memory B cells primed by recently circulating H1N1 seasonal strains were comparably blind to HA1 protective epitopes on pandemic H1, or instead had sufficient binding avidity for vaccine subunit antigens to be activated and to differentiate into plasma cells secreting HI and MN antibodies.

In order to answer some of these questions, we conducted a clinical study where seven young adults received a single dose of the monovalent MF-59-adjuvanted vaccine against 2009 A/H1N1 pandemic influenza (Focetria®). Antibody, CD4+ T cell, plasmablast and memory B cell responses to pandemic A/H1N1 and seasonal antigens were analyzed in parallel. By looking at the kinetic and functional profile of antibody and cell-mediated responses, we found evidence suggestive that CD4+ T cells and memory B cells primed by seasonal H1N1 antigens circulating prior to 2009 were both recruited in the response to pandemic A/H1N1 vaccination and associated with the fast rise of HI and MN antibodies.

2. Subjects and methods

2.1. Study design

This Phase IV, open-label, single-site study (EudraCT number 2009-017141-58) was sponsored by OPERA CRO srl and conducted at the 'Azienda Ospedaliera Universitaria Senese' in Siena, Italy, from January to August 2010. The study's primary objective was the parallel assessment of antibody and cell-mediated immune responses to one dose of egg-derived, inactivated, monovalent subunit vaccine adjuvanted with MF59 (Focetria®) against pandemic A/H1N1 influenza virus produced by Novartis Vaccines and Diagnostics, Siena, Italy. The vaccine contained 7.5 μg of HA per 0.5 ml dose and the adjuvant MF59 (9.75 mg of squalene, 1.17 mg polysorbate 80, and 1.17 mg sorbitan trioleate). Subjects eligible for enrollment were healthy adults between 18 and 60 years of age. Although the protocol was not designed or powered to draw conclusions regarding safety and tolerability, these variables were monitored and recorded. This study was conducted in accordance with the Declaration of Helsinki. The protocol and all the documents related to the study were reviewed and approved by the Ethics Committee of the 'Azienda Ospedaliera Universitaria Senese'. Since the study started when the pandemic season had already reached its peak, subjects with history of recent influenza-like symptoms, or who received seasonal vaccination within 2 weeks prior to day 1, or who were willing to receive it during the 2 weeks following day 1 were excluded from the enrollment. Recruitment was low, consistent with the low rate of vaccinated people registered in Italy in the same period [17]. Out of the 60 subjects planned for enrollment, only 7 subjects presented, provided signed informed consent, and were enrolled in the study. All enrolled subjects (mean age: 42.9 ± 12.2), received one dose of Focetria®, administered intramuscularly, at day 1. Blood samples (approximately 70 ml) from each subject were collected in sodium-heparin tubes at day 1 (before vaccine administration) 8, 22, and 202.

2.2. PBMC and plasma preparation

Blood was collected in sodium-heparin vacutainers. Plasma and PBMC samples were isolated within 6 h after bleeding by centrifugation over Ficoll gradient (Ficoll–Paque, GE Healthcare, Milano, Italy). PBMC were washed twice in Hanks buffered salt solution and cryo-preserved in liquid nitrogen until use. Plasma were frozen and stored at −20 °C until use. In each assay PBMC and plasma samples from all visits were tested simultaneously against all indicated antigens.

2.3. Titration of antibodies by ELISA, hemagglutinin inhibition and micro-neutralization assays

Circulating IgM and IgG antibodies specifically binding to pandemic and seasonal subunit antigens were performed by ELISA. Egg-derived mono bulk subunit antigens (Novartis Vaccines and Diagnostics, Siena, Italy) were absorbed to Maxisorp 96 well ELISA plates (Nunc, Thermo Scientific, Roskilde, Denmark) by adding 100 μl/well of PBS (Invitrogen, Milan, Italy) containing HA at 1 μg/ml (H1N1 subunit from either A/California/07/09 or A/Brisbane/59/07, or at 3 μg/ml (H3N2 subunit from A/Brisbane/10/07) for 16–20 h at 4 °C. Unspecific binding sites were saturated with 200 μl/well of PBS 3% polyvinylpyrrolidone (Serva Electrophoresis, Heidelberg Germany) for 2 h at 37 °C. Plasma samples and standards were added in serial 2-fold dilutions made in 1% BSA in PBS with 0.05% Tween-20 and incubated 2 h at 37 °C. Antigen-specific antibodies of the IgM and IgG class were revealed by an alkaline phosphatase-conjugated goat anti-human IgG, or mouse anti-human IgM antibodies (diluted 1:4000 or 1:2000, respectively), followed by p-nitrophenylphosphate (all detection reagents were from Sigma–Aldrich, St. Luis MO, US). Titers of antigen specific antibodies were determined against internal reference sera (pre-selected to give an $OD_{405\,nm} = 0.5$ in antigen specific ELISA when assessed at the 1:1000 dilution) and expressed as relative ELISA units.

Hemagglutinin inhibition and micro-neutralization assays were performed as described [18] and performed against the pandemic vaccine homologous virus X-179A (expressing HA and NA from the A/California/7/2009 strain), or against 100 $TCID_{50}$ of the 2008–2010 seasonal vaccine strains IVR148 and IVR147 (expressing HA and NA from the A/Brisbane/59/07 and A/Brisbane/10/07 influenza strain, respectively). Plasma samples were analyzed in duplicate and in serial two-fold dilutions starting from an initial dilution of 1:10 to determine absolute end-point titers. Negative samples were assigned a titer of 1:5.

2.4. ICS FACS analysis of CD4+ T cell responses

Antigen specific CD4+ T cell responses were assessed as previously described [1]. Antigens used for PBMC stimulation were egg-derived mono bulk subunit (Novartis Vaccines and Diagnostics) from A/California/07/2009 (pandemic H1N1), A/Brisbane/59/04 (seasonal H1N1) strains, A/Brisbane/10/04 (H3N2), or A/Vietnam/1194/2004 (H5N1). Stimulated samples were stained with the LIVE/DEAD aqua viability marker (Invitrogen), treated with cytofix/cytoperm (BD Biosciences, Milan, Italy) and stained with V450–anti–hCD3 (UCHT1), APC-H7–anti–hCD4 (SK3), PerCP Cy5.5–anti–hIFN–γ, Alexa488–anti–hIL–2 and