Experimental Gerontology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Responders and non-responders to influenza vaccination: A DNA methylation approach on blood cells

Noémie Gensous[a], Claudio Franceschi[a,b,c], Bonnie B. Blomberg[g], Chiara Pirazzini[c], Francesco Ravaioli[a], Davide Gentilini[d], Anna Maria Di Blasio[d], Paolo Garagnani[a,e,f,g,h,*], Daniela Frasca[i,1], Maria Giulia Bacalini[c,1]

[a] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy
[b] Interdepartmental Center "L. Galvani", University of Bologna, Bologna, Italy
[c] IRCCS Institute of Neurological Sciences, Bologna, Italy
[d] Istituto Auxologico Italiano IRCCS, Cusano Milanino, Milan, Italy
[e] Center for Applied Biomedical Research (CRBA), St. Orsola-Malpighi University Hospital, Bologna, Italy
[f] Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Huddinge University Hospital, S-141 86 Stockholm, Sweden
[g] Institute of Molecular Genetics (IGM)-CNR, Unit of Bologna, Bologna, Italy
[h] Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopaedic Institute, Bologna, Italy
[i] Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, USA

ARTICLE INFO

Keywords:
DNA methylation
Influenza vaccination
Aging
Immunosenescence

ABSTRACT

Several evidences indicate that aging negatively affects the effectiveness of influenza vaccination. Although it is well established that immunosenescence has an important role in vaccination response, the molecular pathways underlying this process are largely unknown. Given the importance of epigenetic remodeling in aging, here we analyzed the relationship between responsiveness to influenza vaccination and DNA methylation profiles in healthy subjects of different ages. Peripheral blood mononuclear cells were collected from 44 subjects (age range: 19–90 years old) immediately before influenza vaccination. Subjects were subsequently classified as responders or non-responders according to hemagglutination inhibition assay 4–6 weeks after the vaccination. Baseline whole genome DNA methylation in peripheral blood mononuclear cells was analyzed using the Illumina® Infinium 450 k microarray. Differential methylation analysis between the two groups (responders and non-responders) was performed through an analysis of variance, correcting for age, sex and batch. We identified 83 CpG sites having a nominal p-value < .001 and absolute difference in DNA methylation of at least 0.05 between the two groups. For some CpG sites, we observed age-dependent decrease or increase in methylation, which in some cases was specific for the responders and non-responders groups. Finally, we divided the cohort in two subgroups including younger (age < 50) and older (age ≥ 50) subjects and compared DNA methylation between responders and non-responders, correcting for sex and batch in each subgroup. We identified 142 differentially methylated CpG sites in the young subgroup and 305 in the old subgroup, suggesting a larger epigenetic remodeling at older ages. Interestingly, some of the differentially methylated probes mapped in genes involved in immunosenescence (*CD40*) and in innate immunity responses (*CXCL16*, *ULK1*, *BCL11B*, *BTC*). In conclusion, the analysis of epigenetic landscape can shed light on the biological basis of vaccine responsiveness during aging, possibly providing new appropriate biomarkers of this process.

1. Introduction

Influenza is an important public health challenge in our countries, with yearly epidemics responsible of significant mortality, morbidity and loss of productivity (Paules and Subbarao, 2017). Specific populations such as very young children, individuals aged 65 years and

* Corresponding author at: Alma Mater Studiorum – University of Bologna, Department of Experimental, Diagnostic and Specialty Medicine, Via San Giacomo 12, 40126 Bologna (BO), Italy.
E-mail addresses: claudio.franceschi@unibo.it (C. Franceschi), bblomber@med.miami.edu (B.B. Blomberg), chiara.pirazzini5@unibo.it (C. Pirazzini), francesco.ravaioli2@unibo.it (F. Ravaioli), a.diblasio@auxologico.it (A.M. Di Blasio), paolo.garagnani2@unibo.it (P. Garagnani), dfrasca@med.miami.edu (D. Frasca), mariagiuli.bacalini2@unibo.it (M.G. Bacalini).
[1] Co-senior authorship.

https://doi.org/10.1016/j.exger.2018.01.019
Received 30 September 2017; Received in revised form 20 December 2017; Accepted 16 January 2018

older, or subjects with pre-existent conditions (immunocompromised states, cardiovascular or cerebrovascular diseases, diabetes, chronic respiratory failure, pregnancy) are particularly vulnerable to this infection and at greater risk for complications.

Vaccination is the most effective method to prevent influenza infection. Annual vaccination with an injectable trivalent inactivated vaccine is recommended, especially for individuals aged 65 years or older. However, the protection delivered by these vaccines is incomplete. Rates of protective immune response to vaccination are frequently low in vaccinated subjects, with worsened responses in older adults (Jefferson et al., 2010; Osterholm et al., 2012).

Part of the poor vaccine efficacy in the elderly is due to immunosenescence (Haralambieva et al., 2015; Kennedy et al., 2016; Targonski et al., 2007) but molecular pathways associated with impaired vaccine responses remain incompletely understood. Identification of the mechanisms associated with the development of a protective immunity is of central importance in vaccinology, in order to improve our capacity to predict response to vaccination or develop potential interventions to improve the immune responses.

To date, several studies have been conducted to identify genome-wide changes in transcriptional profiles that correlate with clinical response to influenza vaccination (Bucasas et al., 2011; Nakaya et al., 2011, 2015; Obermoser et al., 2013; Thakar et al., 2015; Tsang et al., 2014; Zhu et al., 2010), by assessing genome-wide gene expression with microarrays before and/or after vaccination of subjects. These molecular signatures, associated with better antibody responses, were frequently enriched in immune pathways, especially with type I interferon signaling, antigen presentation pathways or B-cell proliferation. Thakar et al. identified a dysregulation in this gene signature in older adults, especially in frail subjects who were non-responders to vaccination (Thakar et al., 2015). Other large-scale profiling studies have tried to identify further relevant biomarkers that could predict vaccine response: in this attempt, Furman et al. identified nine immunological baseline predictors of protective immunity, with two of these variables involved in apoptosis (Furman et al., 2013). Finally, models integrating and combining transcriptomic data with additional data types to predict response to vaccination have been developed recently (Tsang et al., 2014; Zimmermann et al., 2017).

While transcriptomic data have been deeply studied in this field, few reports have been published regarding epigenetic aspects. DNA methylation has an important role in several biological processes, especially in aging (Sen et al., 2016), and is therefore an interesting candidate to be investigated. Furthermore, DNA methylation measures tend to be more stable than transcriptomic data within the short period (days-weeks) and are more reproducible from a technical point of view. Lu et al. discovered two relevant epigenetic variations in poor-responders to the vaccine directed against Hepatitis B virus (Lu et al., 2014). Concerning influenza vaccine, one recent study has identified numerous CpG sites showing associations with gene expression and other ones associated with the induction of the humoral immune response (Zimmermann et al., 2016).

To complete these findings and evaluate the effect of age on vaccination response, here we investigated baseline (that is, immediately before vaccination) genome-wide DNA methylation in peripheral blood mononuclear cells (PBMC) from 44 healthy donors, ranging from 19 to 90 years, who received influenza vaccination and were classified as responders and non-responders according to hemagglutination inhibition assays (HIA) after 28 days.

2. Materials and methods

2.1. Participants

Participants (age range: 19–90 years) were recruited at the University of Miami Miller School of Medicine. Experiments were conducted using peripheral blood. Enrolled participants received the influenza vaccine in the pandemic season 2009 and in the season 2010–2011. Participants enrolled in the pandemic 2009 season received the subunit vaccine containing the A/California/7/2009 (H1N1) strain, whereas those enrolled in the 2010–2011 season received the Trivalent Inactivated influenza vaccine containing the following viral strains: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008. Whole blood samples were collected immediately before vaccination. PBMC were collected using Vacutainer CPT tubes (BD 362761). Cells were washed and cryopreserved. Appropriate signed informed consent was obtained from each subject prior to enrollment. The study was approved with IRB protocol #20070481. Each participant was asked questions regarding demographics, health behaviors, presence of symptoms associated with inflammatory conditions or respiratory infections at the time of enrollment. No one reported subclinical inflammatory conditions and/or had respiratory tract infections at the time of enrollment, nor was on any anti-inflammatory treatment or on medications known to alter the immune response. Participants were excluded if they had diseases known to alter the immune response.

2.2. Assessment of response to vaccination

Immunogenicity of influenza vaccine in subjects was assessed by hemagglutination inhibition assays (HIA). For this purpose, blood samples were collected immediately before vaccination (baseline) and 4–6 weeks after to evaluate the in vivo response and identify responders and non-responders. Responders had at least a 4-fold increase in the reciprocal of the titers in response to the whole vaccine, which was the A/California/7/2009 (H1N1) strain in the 2009 season and the Trivalent Inactivated Influenza vaccine (containing A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), B/Brisbane/60/2008) in the 2010–2011 season. Briefly, sera were pretreated with receptor destroying enzyme (RDE, Denke Seiken Co Ltd) for 20 h at 37 °C; in order to inactivate this enzyme, sera were then heated at 56 °C for 60 min. Two-fold serial dilutions were done; 25 µL of diluted sera were incubated with an equal volume of 4 HA units of the 2009 vaccine or of the 2010–2011 vaccine, for 1 h at room temperature and then 50 µL of a 1.25% suspension of chicken red blood cells were added. After 2 h of incubation at room temperature titers were determined.

2.3. Genome-wide DNA methylation analysis

Genome-wide DNA methylation analysis was performed on PBMC collected immediately before vaccination (baseline) and cryopreserved. DNA methylation patterns are generally stable, highly reproducible and only slightly affected by freezing (Bulla et al., 2016). Total genomic DNA was extracted from PBMC using the AllPrep DNA/RNA Mini kit (Qiagen), according to manufacturer's instructions. DNA concentrations were determined using NanoDrop spectrophotometer. DNA was bisulfite-converted using the EZ DNA Methylation Kit (Zymo Research Corporation®) and analyzed on the Infinium HumanMethylation450 BeadChip (Illumina®) following manufacturer's instructions (Bibikova et al., 2011). Arrays were scanned by HiScan (Illumina®) and signal intensities were extracted from *.idat* files using the *minfi* Bioconductor package (Aryee et al., 2014). Data were normalized using the preprocess Quantile function of the package *minfi*. Probes on the X and Y chromosomes were removed, as well as probes associated to a SNP. Identification of CpG sites with differential methylation between responders and non-responders to influenza vaccination was performed through an analysis of variance (ANOVA) model, correcting for sex and batch. CpG sites differentially methylated between responders and non-responders were defined as having a nominal p-value inferior to 0.01 and an absolute difference between values of responders and non-responders of at least 0.05. Figures were generated using R.