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Quantitative profiling reveals minor changes of T cell receptor repertoire in response to subunit inactivated influenza vaccine

Anastasiia L. Sycheva [a,1], Mikhail V. Pogorelyy [a,1], Ekaterina A. Komech [a,b], Anastasia A. Minervina [a], Ivan V. Zvyagin [a,b], Dmitriy B. Staroverov [a,b], Dmitriy M. Chudakov [a,b,c,d], Yuri B. Lebedev [a,b], Ilgar Z. Mamedov [a,b,*]

[a] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Miklukho-Maklaya 16/10, 117997 Moscow, Russia
[b] Pirogov Russian National Research Medical University, 117997 Moscow, Russia
[c] Skolkovo Institute of Science and Technology, Skolkovo 143025, Russia
[d] Central European Institute of Technology, Brno 60177, Czech Republic

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ABSTRACT

Vaccination against influenza is widely used to protect against seasonal flu epidemic although its effectiveness is debated. Here we performed deep quantitative T cell receptor repertoire profiling in peripheral blood of a healthy volunteer in response to trivalent subunit influenza vaccine. We did not observe significant rebuilding of peripheral blood T cell receptors composition in response to vaccination. However, we found several clonotypes in memory T cell fraction that were undetectable before the vaccination and had a maximum concentration at day 45 after vaccine administration. These cells were found in lower concentration in the course of repertoire monitoring for two years period. Our observation suggests a potential for recruitment of only a limited number of new T cells after each seasonal influenza vaccination.

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1. Introduction

Influenza virus remains one of the most widespread respiratory infections in humans causing annual epidemics and substantial mortality and cure costs [1–5]. Certain groups of people are at higher risk for mortality and secondary complications, including the elderly, infants, and people with chronic diseases. Although the protective effect of influenza vaccines is less pronounced [4,6–9] compared to other widely used vaccines it is still one of the most efficient ways to protect against flu infection. The annual influenza vaccine includes three or four influenza virus A and influenza virus B strains circulating last year and predicted for the next year. The two most widely used types of influenza vaccines are inactivated vaccines consisting of isolated viral proteins (hemagglutinin (HA) and neuraminidase (NA)) or split virions and live attenuated influenza vaccine (LAIV) containing the attenuated virus [1]. Additionally, vaccines containing conservative fragments of viral proteins such as peptide M2e and HA-stalk or internal proteins are proposed for universal influenza vaccine development. Some of them are currently under clinical trials [10].

The adaptive immune system response to influenza virus or to the administered vaccine has been studied for many years. One of the key components of the immune response to the virus is the neutralizing antibodies targeting variable hemagglutinin glycoprotein [11–13]. These antibodies prevent binding of virus particles to the cell surface. The titer of such antibodies is used to evaluate vaccines efficiency and protective strength [14,15]. Other non-neutralizing antibodies against surface proteins were also shown to correlate with protection against the virus, however the exact mechanism of their function is still poorly understood [13,16,17]. There is an evidence [18,19] that these antibodies have more cross-reactivity and give protection against a broad range of influenza virus strains unlike highly strain specific anti-HA neutralizing antibodies.

The role of T cell response in protection against influenza virus is more complex and the data is highly controversial. Pioneer studies demonstrated that the number of cytotoxic T lymphocytes (CTL) correlates with viral clearance [20] after infection. The majority of healthy adults have influenza-reacting CD8 and CD4 T cells in peripheral blood [21,22]. In one of the recent papers the leading

* Corresponding author at: Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Miklukho-Maklaya 16/10, 117997 Moscow, Russia.
E-mail address: Imamedov@mx.ibch.ru (I.Z. Mamedov).
[1] These authors contributed equally to this work.

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role of CD4 T cells in protection against influenza virus in seronegative donors was reported [23]. Although the data on T cell response to influenza vary it is generally accepted that virus-specific T cells protect from a broad range of strains and exhibit substantial cross-reactivity [24–26]. Accordingly, the influenza-specific T cells are shown to recognize conservative fragments of NA, M1, NP, PB1 as well as HA, NA and M2 influenza virus proteins [22,23,27–29]. It is believed that T cell response should be intensively studied to identify new targets for universal and more effective influenza vaccines [30].

The data on T cell response to vaccination is also diverse [31–34]. One of the first works by McMichael and his colleagues [35] reported increased number of cytotoxic T lymphocytes (CTL) after inactivated whole virus vaccine administration. Subunit vaccine in the same study demonstrated patient specific reaction: in some patients, the number of CTL increased, in the other groups it decreased or did not change. In more recent studies the minor increase in the number of influenza-specific cytokine secreting CD4+ T cells was reported after admission of split and subunit vaccine with and without adjuvant [36–39]. CD4+ cells expansion in response to the influenza vaccine was also detected by influenza virus peptide-MHC tetramer complex staining [40].

Although the bulk T cell response to influenza vaccines is proven, the repertoires of T cell receptors reacting to vaccine was not characterized. Here we applied the quantitative NGS based technique to characterize the repertoires of T cells before and after vaccination and to track individual T cell clones in a healthy volunteer.

2. Materials and methods

2.1. Blood sample collection

All the studies were performed according to the declaration of Helsinki and after the positive decision of the local ethical committee. Informed consent was obtained from the volunteer (58 years old). The volunteer was annually vaccinated by Influvac® subunit influenza vaccines for several years before this study. During this three-year study, the volunteer received different Influvac® subunit vaccine three times: for 2014/2015, 2015/2016 and 2016/2017 seasons. All vaccines shared A/California/7/2009 (H1N1)pdm09-derived strain. 12 ml of peripheral blood (PB) were collected for each time point and divided into two full PBMC and specific T cell fractions (See Fig. 1 for details). PBMCs were isolated by Ficoll-Paque (Paneco, Russia) density gradient centrifugation according to standard protocol.

CD4 and CD8 T cells were isolated from PBMCs using the CD4+ and CD8+ positive selection kit (Invitrogen, USA) according to the manufacturer's protocol. CD8 T cells were isolated from CD4 depleted samples to maximize the cell yield. "Non-naive cells" fraction was isolated by magnetic separation using Naive CD8+ T Cell isolation kit (Myltenyi, USA). Positively selected cells include memory and effector T lymphocytes but not naive T cells. Obtained fractions and bulk PBMC (full fractions) were used for total RNA isolation with the Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol.

2.2. TCR β cDNA library preparation

The TCR β-chain cDNA library preparation was performed as previously described [41,42]. In brief, the cDNA first strand was produced from the total RNA using the SmartScribe revertase (Clontech, USA) and universal primers specific for the TCR β-chain constant (C) segment. SMART oligonucleotides were used to generate universal 5′ ends and to introduce unique molecular identifiers (UMIs). After the cDNA synthesis, two steps of PCR amplification were performed to amplify the cDNA and introduce Illumina adapters at the ends (see Supplementary Table 1 for all primer sequences). The PCR product was purified using the Qiagen PCR purification kit according to the manufacturer's protocol.

2.3. Next generation sequencing

cDNA libraries were sequenced on the Illumina HiSeq (2x100nt). The total number of sequencing reads for each sample is shown in Supplementary Table 2. Raw data is available in the Sequence Read Archive at https://trace.ncbi.nlm.nih.gov/Traces/sra/, accession number SRP111073.

2.4. Raw data preprocessing

Raw sequencing data files were preprocessed with MiGEC [43], sequencing reads were clustered by UMIs. Then sequences were processed with MiXCR [44] to extract TCR CDR3 sequences, determine V, D and J genes and build clonotypes. For the numbers of UMIs and clonotypes after filtering see Supplementary Table 2.

2.5. Statistical analysis

For statistical analysis of clonal concentration changes edgeR package [45] was applied. edgeR package is used to analyse multiple types of count data from NGS studies, including RNA-seq for differential gene expression, differential splicing and even bisulfite sequencing. Reproducibility of counts observed in RNA-seq experiments is vary between technical (the same library sequenced several times) and biological replicates (libraries prepared from different samples of cells). In the first case counts in replicates are very reproducible and follow Poisson distribution, in second case there is much additional noise, introduced by noisy amount of RNA in each cell and library preparation procedures. edgeR requires biological replicates to fit negative binomial model for this additional biological noise. In this study two biological replicates for each time point was used to learn noise model, no data was missing. Standard deviation-mean relationship for our dataset is shown at SI. Fig. 1, trended dispersion estimate was found adequately explaining variance on individual clonotypes in data, there are no clonotypes with tagwise dispersion estimates deviating much from the trend line. Testing for difference in clonal concentrations between pairs of time points was performed with exact test based on qCML (quantile-adjusted conditional maximum likelihood) method realised in edgeR software (as described in point 2.9 in edgeR user manual).

Fisher's exact test and Barnard's test (exact unconditional test) were used for comparative analysis of CD4/CD8 phenotype distribution. Search for similar amino acid sequence was done using Levenshtein distance estimation ("stringdist" package).

3. Results

One healthy volunteer was vaccinated by trivalent inactivated subunit influenza vaccine (Influvac, see Section 2). Peripheral blood (PB) samples were collected 14 days prior (day −14), at the day of vaccination (day 0) and days 5, 12, 45 after vaccine administration. For the two-year period after day 45 donor was vaccinated annually with the seasonal vaccine of the same type but with different strain composition. Two additional PB samples were collected three months prior and 14 days after the last vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated from the collected samples and used for TCR beta cDNA library preparation and high-throughput Illumina sequencing [41,42,46]. TCR clono-

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