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Cytomegalovirus-associated accumulation of late-differentiated CD4 T-cells correlates with poor humoral response to influenza vaccination

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ABSTRACT

Influenza vaccination is less effective in the elderly compared to the young. Studies that have attempted to identify immune parameters correlating with satisfactory vaccine responses have yielded inconclusive results. Here, we correlate the distribution of different circulating CD4+ and CD8+ T-cell phenotypes with the humoral response to vaccination with Intanza, an intradermal seasonal vaccine, in 54 individuals of different ages. Subjects were stratified according to age (below or over 60) and presence of a latent infection with Cytomegalovirus (CMV). CMV-seropositivity was significantly associated with a lower response rate to the vaccine in people over but not below 60 yr of age. Unlike reported data, late-differentiated (CD45RA+CCR7−CD27−CD28−) CD4+, but not CD8+ T-cells associated with a poorer vaccine response. Thus, latent CMV infection has a deleterious effect on influenza antibody responses in the elderly, which might be mediated through CD4 T-cells lacking CCR7, CD27 and CD28 and re-expressing CD45RA.

1. Introduction

Seasonal influenza is a disease with serious clinical and economic burdens, estimated to be the cause of an average of 226,000 hospitalizations and 36,000 deaths per season between 1979 and 2001 in the United States, with 90% of deaths occurring in individuals over the age of 65 [1,2]. Annual influenza vaccination is the most effective method for preventing influenza and its complications [3]. However, exactly the group most susceptible to influenza morbidity and mortality, the elderly, does not respond as well as the young to vaccination. While in the young, the clinical vaccine efficacy is estimated at 70–90%, this corresponds to only 17–53% in the elderly, depending on the vaccine matching with the viruses in circulation [4]. This is widely believed to be due to immunosenescence, the diminished state of the immune system observed in the elderly [5,6].

The specific mechanisms responsible for the decreased ability of the elderly to respond to influenza vaccination are still poorly understood. CD8+ T-cells lacking the costimulatory receptor CD28 have been associated with poor humoral and cellular response to influenza vaccination in the elderly [7–9]. CD28 down-regulation is the result of several rounds of T-cell division in response to antigenic challenge. Thus, CD8+ T-cells specific for chronic antigens, such as human immunodeficiency virus (HIV), cytomegalovirus (CMV) and to a lesser extent Epstein–Barr virus (EBV) lack the expression of this receptor [10–12]. CMV is an almost-ubiquitous β-herpes virus present in 30–90% of the population in developed countries with a rising seroprevalence with increasing age [13]. Although asymptomatic in immunocompetent individuals, it has an enormous impact on the immune system of the host – more than 30% of CD4+ and CD8+ T-cells of a healthy middle-aged individual can be specific for this virus [14]. A latent CMV infection is associated with lower levels of naïve T-cells and accumulation of memory T-cells, both hallmark features of immunosenescence [15–18]. This has led to the increasingly accepted notion that CMV accelerates T-cell immunosenescence [19]. Accordingly, CMV has been associated with poor humoral response to influenza vaccination in the elderly in some [20,21], but not all studies [22].

Thus, if and how CMV-seropositivity and the accumulation of CD28− late-differentiated T-cells associated with it contribute to poor vaccination outcome in the elderly is still unclear. The few studies demonstrating a negative correlation between CD28−CD8+ T-cells and poor vaccination outcome [7–9] did not take CMV-serostatus into account, thus failing to demonstrate a direct link

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between accumulation of these cells and poor responsiveness to influenza vaccine.

Here we have correlated the detailed phenotype of circulating CD4+ and CD8+ T-cell subsets with humoral responses in young and old individuals receiving Intanza, an intradermal influenza vaccine specially designed for the elderly [23]. Our data demonstrate a negative impact of the presence of CD4+ but not CD8+ T-cells with a more late-differentiated phenotype on responsiveness to the vaccine in individuals over the age of 60. This suggests that the negative impact of CMV on vaccination outcome, observed in our study, might be mediated through CD4 T-cells lacking the expression of CCR7, CD27 and CD28 and re-expressing CD45RA.

2. Materials and methods

2.1. Study population and design

The current study was embedded in an open-label uncontrolled multicenter phase III trial (UTN U1111-1112-2795) that evaluated the humoral immunogenicity and safety of the Northern Hemisphere 2010–2011 formulation of an intradermal inactivated split-virion influenza vaccine (Intanza®, Sanofi Pasteur) in adults (18–59 yr) and elderly (>60 yr), as required for marketing authorization. Participants at the University of Antwerp were invited after the last visit of the clinical trial (June–July 2010) to take part in a subsequent study looking at CMV status and cellular phenotypes. Approval for this subsequent study was obtained from the Ethical Review Board of the Antwerp University Hospital. Both the clinical trial and the subsequent study were performed according to ICH/GCP guidelines.

Exclusion criteria for enrollment in the clinical trial were contra-indications for influenza vaccination (such as systemic hypersensitivity to a vaccine component), pregnancy or breastfeeding, history of pandemic H1N1 infection or vaccination, history of influenza vaccination within the preceding 6 months (12 months for adjuvanted vaccines), immunodeficiency, receipt of blood products in the past 3 months, seropositivity for HIV, hepatitis B or C, and risk factors that might affect compliance to trial procedures such as chronic illness. At the time of the first vaccine administration (D0), volunteers had to be free of acute illness or infection, and were not allowed to have received or plan receipt of any other vaccine in a period starting 4 weeks before until 3 weeks after trial vaccination. After blood sampling, participants were vaccinated intradermally in the opposite arm with a vaccine containing antigens to A/California/7/2009 (H1N1); A/Perth/16/2009 (H3N2); and B/Brisbane/60/2008 (Barr, Vaccine 2009). According to the marketing authorization, 18–59 yr-olds received 9 μg of each HA strain whereas participants ≥60 yr received 15 μg. A second visit (D21) was scheduled 21 d (±1 d) after the first, for a second blood draw and safety follow-up. D0 and D21 blood samples were used for antibody response assessment only and were centrifuged within 2 h and stored at −20 °C until use.

Participants who agreed to take part in the subsequent study ($n = 55$ of a total of 66) were invited for a third blood draw within two weeks after D21, for immune phenotyping and CMV serology. Peripheral blood mononuclear cells (PBMC) were isolated within 20 h by ficoll-paque density gradient centrifugation and cryopreserved at −196 °C. A separate sample for CMV antibody assessment was centrifuged within 2 h and frozen at −20 °C until further processing.

2.2. Assessment of humoral response to influenza vaccine

The antibody titers to each vaccine strain were assessed by a standard haemagglutination inhibition assay (HIA) at Sanofi-Pasteur's laboratory (Global Clinical Immunology [GCI], Swiftwater, PA, USA). The antibody titer was defined as the highest reciprocal dilution inducing complete haemagglutination inhibition (HI) [24]. Each sample was tested in duplicate and the final titer was the geometric mean of the duplicates. Lower and upper limits of quantification were set at 10 and 10,240 respectively. For the B-strain, the HIA used ether-treated split antigen to enhance sensitivity [25].

A positive humoral response was defined as an at least 4-fold antibody titer rise (from D0 to D21) against at least two of the three vaccine strains.

2.3. Assessment of CMV-serostatus

CMV-specific IgG and IgM were measured using Cobas® assays (Roche Diagnostics) [26]. IgM results were qualitative only. All samples were tested at the microbiology laboratory of the University Hospital of Antwerp.

2.4. T-cell analysis

Flow cytometry experiments were performed as described previously [27]. Briefly, PBMC were thawed and treated with human immunoglobulin GAMUNEX (Bayer, Leverkusen, Germany), and ethidium monoazide (EMA) (Invitrogen, Karlsruhe, Germany). Cells were first stained indirectly with anti-KLRG-1 primary antibody (kindly provided by Prof. H-P Pircher, Freiburg, Germany) and Pacific Orange-conjugated anti-mouse IgG (Invitrogen). After blocking with mouse serum (Chemicon/Millipore, Schwalbach, Germany) monoclonal antibodies CD3–Alexa Fluor 700, CD4–PerCP, CD8–APC-H7, CCR7–PE-Cy7, CD45RA–V450, CD28–PE (BD Biosciences, Heidelberg, Germany), CD27–APC (BioLegend, San Diego, CA), and CD57–FITC (Immunotools, Freiburg, Germany) were added. Cells were washed and analyzed immediately on an LSR II cytometer with FACSDiva software (BD Biosciences). The spectral overlap between all channels was calculated automatically by the BD FACSDiva software, after measuring negative and single-color controls. Data were analyzed using FlowJo software (Tree Star, Portland, OR).

For data analysis, EMA-positive dead cells were excluded. In the viable gate, lymphocytes were gated according to their size and granularity. T-cells within the lymphocyte gate were characterized as CD3+ cells. Differentiation stages of T-cells were characterized according to surface expression of CD45RA, CCR7, CD27 and CD28, according to previously described models [11,28] (Supplementary Fig. 1). Positive and negative populations were gated based on fluorescence-minus-one (FMO) controls for each fluorochrome.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.11.041.

2.5. Intracellular cytokine staining

PBMC were thawed and allowed to rest for 8 h in X-Vivo15 medium (Cambrex) at 1×10^6 ml at 37 °C. The cells were then incubated overnight with 50 ng/ml PMA (Sigma–Aldrich, Munich, Germany), 750 ng/ml Ionomycin (Merck, Darmstadt, Germany) and 1 μl/ml Golgi-Plug (BD Biosciences). Following fixation and permeabilisation using Cytofix–Cytoperm solution (BD Biosciences), the cells were stained with CD3–Qdot 655 (Invitrogen), CD4–Pacific Blue, IL-2-Alexa Fluor 700, IL-5-PE, TNF-FITC (BioLegend), CD8–APC-H7, IFN-γ-PE-Cy7 (BD Biosciences), IL-10-APC (Miltenyi Biotec, Bergisch Gladbach, Germany), and IL-17-PerCP-Cy5.5 (eBioscience, Frankfurt, Germany). EMA was used to exclude dead cells. Samples were measured directly using the BD LSR-II as described above. Production of different cytokines by T-cells in a