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Influenza vaccine-mediated protection in older adults: Impact of influenza infection, cytomegalovirus serostatus and vaccine dosage

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ABSTRACT

Age-related changes in T-cell function are associated with a loss of influenza vaccine efficacy in older adults. Both antibody and cell-mediated immunity plays a prominent role in protecting older adults, particularly against the serious complications of influenza. High dose (HD) influenza vaccines induce higher antibody titers in older adults compared to standard dose (SD) vaccines, yet its impact on T-cell memory is not clear.

The aim of this study was to compare the antibody and T-cell responses in older adults randomized to receive HD or SD influenza vaccine as well as determine whether cytomegalovirus (CMV) serostatus affects the response to vaccination, and identify differences in the response to vaccination in those older adults who subsequently have an influenza infection.

Older adults (≥ 65 years) were enrolled (n = 106) and randomized to receive SD or HD influenza vaccine. Blood was collected pre-vaccination, followed by 4, 10 and 20 weeks post-vaccination. Serum antibody titers, as well as levels of inducible granzyme B (iGrB) and cytokines were measured in PBMCs challenged *ex vivo* with live influenza virus. Surveillance conducted during the influenza season identified those with laboratory confirmed influenza illness or infection.

HD influenza vaccination induced a high antibody titer and IL-10 response, and a short-lived increase in Th1 responses (IFN-γ and iGrB) compared to SD vaccination in PBMCs challenged *ex vivo* with live influenza virus. Of the older adults who became infected with influenza, a high IL-10 and iGrB response in virus-challenged cells was observed post-infection (week 10 to 20), as well as IFN-γ and TNF-α at week 20. Additionally, CMV seropositive older adults had an impaired iGrB response to influenza virus-challenge, regardless of vaccine dose.

This study illustrates that HD influenza vaccines have little impact on the development of functional T-cell memory in older adults. Furthermore, poor outcomes of influenza infection in older adults may be due to a strong IL-10 response to influenza following vaccination, and persistent CMV infection.

1. Introduction

Older adults are at increased risk of death and complications due to influenza infection (Simonsen et al., 2000; Schanzer et al., 2007), accounting for 90% of influenza-related deaths (Thompson et al., 2010). While vaccinations rates have increased over the years (Gionet, 2015), hospitalizations rates remain unchanged (Thompson et al., 2004).

One of the causes of increased influenza associated morbidity and mortality in older adults is decreasing vaccine effectiveness with age (Goodwin et al., 2006; Simonsen et al., 2007). The current influenza vaccine strategy hinges on inducing an antibody response, but has poor efficacy in older adults. Age-related changes in both B and T-cells are associated with a decline in the antibody response to vaccination (Goronzy et al., 2001; Saurwein-Teissl et al., 2002; Frasca et al., 2010; Frasca et al., 2016). Furthermore, influenza antibody titers have been shown to be a poor correlate of protection in older adults (McElhaney et al., 2006; McElhaney et al., 2009). Cytotoxic T-lymphocyte (CTL) responses have been identified to be protective against influenza disease (La Gruta and Turner, 2014), thus underlining the importance of including measures of the cellular immune response in the assessment of influenza vaccine efficacy (Effros, 2007). Specifically, granzyme B (GrB) activity and IFN-γ:IL-10 ratios in influenza virus-challenged PBMCs have been found to correlate with protection against influenza in older adults (McElhaney et al., 2006; McElhaney et al., 2009; Shahid

Abbreviations: bGrB, baseline granzyme B; CMV, cytomegalovirus; CTL, cytotoxic T-lymphocyte; GMT, geometric mean titer; HA, hemagglutinin; HAI, hemagglutination inhibition assay; HD, high dose; iGrB, inducible granzyme B; ILI, influenza like illness; MLD, minimum level of detection; SD, standard dose

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et al., 2010) and could be useful measures when evaluating vaccine efficacy.

The majority of older adults are seropositive for cytomegalovirus (CMV) (Staras et al., 2006) which may be a confounding factor in evaluating the immune response to influenza vaccination (Frasca et al., 2015; Furman et al., 2015; McElhaney et al., 2015; Haq et al., 2016). Persistent CMV infection has been shown to be a major driver of terminal differentiation of $CD8^+$ T-cells (Derhovanessian et al., 2009; McElhaney et al., 2009). These terminally differentiated $CD8^+$ T-cells express and release the cytolytic mediator, GrB, in the absence of perforin in both the resting and activated state (Zhou and McElhaney, 2011), and can contribute to toxicity in the extracellular space (McElhaney et al., 2012). The presence of high levels of baseline GrB (bGrB) in the resting state of unstimulated T-cells hinders the inducible GrB (iGrB) response to *ex vivo* influenza challenge (McElhaney et al., 2012; Haq et al., 2016) and may result in a compromised response to infection.

Previous studies comparing high dose (HD) and standard dose (SD) influenza vaccine formulations in older adults found significantly higher antibody titers in those receiving HD vaccinations (Couch et al., 2007; Falsey et al., 2009; DiazGranados et al., 2013; DiazGranados et al., 2014) but the impact of vaccine dose on cellular immune response requires further investigation.

Here we present the results of a randomized study comparing SD and HD influenza vaccines in older adults using longitudinal sampling and an *ex vivo* infection model. Changes in cell-mediated immune responses pre- and post-vaccination were measured to determine the impact of vaccine dose, influenza infection, and CMV seropositivity.

2. Materials and methods

2.1. Population

Ethics approval was obtained from local ethics committees: University of Connecticut Health Centre and Health Sciences North (ClinicalTrials.gov Identifier: NCT02297542). Older adults (≥ 65 years of age, n = 106) and young adults (20–40 years of age, n = 19) were recruited through the UConn Center on Aging Recruitment Core (UCARC) and the Health Sciences North Research Institute. Written informed consent was obtained from all study participants. The inclusion criteria for this study required older adults to have received an influenza vaccination in the previous influenza season. Exclusion criteria included: (a) immunosuppressive disorders or medications (including oral prednisone in doses > 10 mg daily); (b) inability to be vaccinated due to a previous significant adverse reaction to influenza vaccine, eggs, latex, or thimerosol, or refusal of vaccination; (c) recipients of influenza vaccination from a community-based program for the approaching influenza season; and (d) pregnancy at week 0 (pre-vaccination).

2.2. Vaccination

Older adults were randomized to receive either the trivalent, split-virus Sanofi Pasteur Fluzone SD vaccine (15 μg of Hemagglutinin (HA) per strain) (n = 53) or Fluzone HD vaccine (60 μg of HA per strain) (n = 53). All young adults received the Fluzone SD vaccine. This study was conducted over the 2014/2015 flu season. The trivalent influenza vaccine in this season consisted of: A/California/7/2009 (H1N1)-like virus, A/Texas/50/2012 (H3N2)-like virus and B/Massachusetts/2/2012-like virus.

2.3. Sample collection

Whole blood samples were collected pre-vaccination (week 0) and also at 4, 10 and 20 weeks post-vaccination. PBMCs were isolated from heparinized blood samples using Ficoll-Plaque Plus (GE Healthcare) gradient purification and transferred to liquid nitrogen for storage. Plasma and serum samples were collected and stored at –80°C.

2.4. Frailty measures

To measure frailty, two measures were used. The Fried Model was used to measure the Frailty phenotype based on the number of deficits: unintended weight loss, tiredness, weak grip strength, slow walking speed, and physical inactivity (Fried et al., 2001). Scores were categorized as follows: 3–5, frail; 1–2, pre-frail; and 0, non-frail. The Frailty Index was determined by assessing 40 validated deficits (McNeil et al., 2012), calculating the sum of deficits then divided by the total deficits considered (Rockwood and Mitnitski, 2011). Individuals with a low Frailty Index (< 0.05) were classified as non-frail, whereas those with an index value of > 0.4 were classified as frail; those with intermediate values were considered to be pre-frail.

2.5. Influenza surveillance

Participants received weekly phone calls during the influenza season and were requested to report any influenza-like illness (ILI) or acute respiratory infection (ARI). ILI was defined by the presence of two respiratory symptoms (cough, sore throat, shortness of breath, and nasal stuffiness) or 1 respiratory and 1 systemic symptom (headache, malaise, oral temperature > 37°C or fever and muscle ache). When reported symptoms met the ILI criteria and were within 5 days of symptom onset, a nasal swab was collected. A laboratory diagnosis of influenza illness was confirmed by the detection of influenza virus by PCR assay of the nasal swab or a 4-fold or greater rise in the antibody titer from pre-vaccination to 4-week post-vaccination.

2.6. Influenza serological analysis

Hemagglutinin-inhibition (HAI) antibody titer assays for A/California/7/2009 (H1N1), A/Texas/50/2013 (H3N2), and B/Massachusetts/2/2012 virus strains were performed using previously described standard methodologies (Webster et al., 2002; Lancaster and Febbraio, 2014).

2.7. CMV serology

The CMV serostatus was determined by testing serum using a CMV IgG ELISA kit (Genesis Diagnostics Inc., Cambridgeshire, UK) according to the manufacturer's instructions.

2.8. Cell stimulation

To measure cellular immune responses, PBMCs were stimulated with live influenza virus as previously described (McElhaney and Gentleman, 2015). Briefly, PBMCs were challenged *ex vivo* with live influenza virus A/Victoria/3/75 (H3N2) or B/Lee/40 (Charles River). Influenza A/H1N1 stimulation was not performed. After a 20-h incubation at 37°C, PBMC lysates and supernatants were collected and frozen at –80°C. Viral strains matching those in the 2014–2015 influenza vaccine were not used as our studies focus on investigating T-cell response, which are primarily targeted against conserved internal or non-structural proteins (Thomas et al., 2006). Further, we have a consistent commercially available source of sucrose gradient-purified influenza virus, which we have shown stimulates similar T-cell responses when compared to seasonal influenza strains (unpublished data).

2.9. Granzyme B assay

GrB activity was measured based on cleavage of the peptide substrate, IEPDpNA (EMD Millipore), which causes a colorimetric change