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Randomized Control Trials

Impact of ageing and a synbiotic on the immune response to seasonal influenza vaccination; a randomised controlled trial

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SUMMARY

Background & aims: Ageing increases risk of respiratory infections and impairs the response to influenza vaccination. Pre- and pro-biotics offer an opportunity to modulate anti-viral defenses and the response to vaccination via alteration of the gut microbiota. This study investigated the effect of a novel probiotic, *Bifidobacterium longum bv. infantis* CCUG 52486, combined with a prebiotic, gluco-oligosaccharide, on the B and T cell response to seasonal influenza vaccination in young and older subjects .

Methods: In a double-blind, randomized controlled trial, 58 young (18–35 y) and 54 older (60–85 y) subjects were supplemented with the synbiotic for 8 weeks. At 4 weeks they were administered with a seasonal influenza vaccine. B and T cell phenotype and responsiveness to *in vitro* re-stimulation with the vaccine were assessed at baseline, 4, 6 and 8 weeks.

Results: B and T cell profiles differed markedly between young and older subjects. Vaccination increased numbers of memory, IgA^+ memory, IgG^+ memory and total IgG^+ B cells in young subjects, but failed to do so in older subjects and did not significantly alter T cell subsets. Seroconversion to the H1N1 subunit in the older subjects was associated with higher post-vaccination numbers of plasma B cells, but seroconversion was less consistently associated with T cell phenotype. B and T cell subsets from both young and older subjects demonstrated a strong antigen-specific recall challenge, and although not influenced by age, responsiveness to the recall challenge was associated with seroconversion. In older subjects, CMV seropositivity was associated with a significantly lower recall response to the vaccine, but the synbiotic did not affect the responsiveness of B or T cells to re-stimulation with influenza vaccine.

Conclusions: Antigen-specific B and T cell activation following an *in vitro* recall challenge with the influenza vaccine was influenced by CMV seropositivity, but not by a synbiotic.

Registered under ClinicalTrials.gov Identifier no. NCT01066377.

1. Introduction

Immunosenescence reduces protection against infections and leads to poor responses to vaccination in older individuals [1]; as a result, influenza is a major cause of mortality in older adults [2,3]. Poor vaccine efficacy against influenza in older individuals is not just a result of impaired antibody production, although this may be a contributing factor. Helper T cells play a vital role in the generation of vaccine-specific antibody production and viral clearance depends on cytotoxic T cells [4]. In fact, cellular immune function may even be better correlated with vaccine protection than the antibody response to influenza vaccination [5]. Repeated antigenic stimulation, activation and differentiation of T cells during ageing causes progressive loss of CD28 and shrinkage of the naïve and early memory cytotoxic T cell compartments [6,7], altering both the quantity and quality of antibodies indirectly [8,9]. Therefore,

Abbreviations: CIRS, cumulative illness rating scale; CMV, cytomegalovirus; Gl-OS, gluco-oligosaccharide; Treg, regulatory T cells; URTI, upper respiratory tract infection.

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understanding the changes that occur in humoral and cell-mediated immunity with ageing is critical for developing strategies to protect against infection and maintain or enhance the response to vaccination.

Previous studies investigating the effects of probiotics on the response to vaccination have mainly focused on antibody production. While some studies have reported a modest effect of probiotics on the antibody response to vaccination in adults, trials in older subjects are largely inconsistent and data are limited [10]. The strain *Bifidobacterium longum bv. infantis* CCUG 52486 was originally isolated from a cohort of very healthy elderly subjects (independent life-style, free of chronic disease, and aged 90 years or over) in Italy as part of the CROWNALIFE EU FP5 project [11]. It has been demonstrated to have particular ecological fitness and antipathogenic effects *in vitro*, and it has immunomodulatory effects which are strongly influenced by the age of the host [12]. Furthermore, this strain has been fully genome sequenced so that genetic traits can potentially be related to biological effects. We recently reported that although a pre- and probiotic combination failed to reverse a marked impairment of the antibody response to influenza vaccination in older subjects, it did tend to improve production of vaccine-specific IgM and IgG in young subjects, but not older subjects, suggesting an age-dependent response to the intervention [13]. However, immunological characterization revealed that the older subjects randomized to the synbiotic had a significantly higher number of senescent ($CD28^{-}CD57^{+}$) helper T cells at baseline compared with those randomized to the placebo. They also had significantly greater tendency for seropositivity to cytomegalovirus (CMV) and higher plasma levels of anti-CMV IgG, which are associated with replicative senescence of T cells [13]. Moreover, higher numbers of $CD28^{-}CD57^{+}$ helper T cells were associated with failure to seroconvert to the Brisbane subunit of the vaccine, strongly suggesting that the subjects randomized to the synbiotic were already at a significant disadvantage in terms of likely ability to respond to the vaccine compared with those randomized to the placebo [13].

In this study, we examine the effects of the synbiotic on antigen-specific B and T cell activation following an *in vitro* vaccine recall challenge. This is important because previous studies have focussed almost entirely on antibody responses to vaccination and there is no information on the effects of pre- or pro-biotics on B and T cell recall responses to vaccination.

2. Methods

2.1. Ethics and trial registration

The study protocol was reviewed and approved by the University of Reading Research Ethics Committee (project number: 10/09) and the National Health Service (NHS) Research Ethics Committee for Wales (10/MRE09/5). The trial was registered with ClinicalTrials.gov (Identifier: NCT01066377) and conducted according to the guidelines laid down in the Declaration of Helsinki.

2.2. Participants

Prior to the influenza season of 2010–2011, young (18–35 y) and older (60–85 y) healthy adults were recruited from the population in and around Reading (UK) through newspaper and poster advertisements, email and radio from June 2010 to March 2011. Inclusion criteria were: a signed consent form, age 18–35 y or 60–85 y, body mass index (BMI) 18.5–30 kg/m^2, good general health, as determined by medical questionnaires and laboratory data from screening blood and urine sample (fasting glucose, erythrocyte sedimentation rate, full blood count, liver function tests, renal profile, dipstick urinalysis), not pregnant, lactating or planning a pregnancy. Exclusion criteria included: allergy to the influenza vaccine, HIV infection, diabetes requiring any medication, asplenia and other acquired or congenital immunodeficiencies, any autoimmune disease, including connective tissue diseases, malignancy, cirrhosis, connective tissue diseases, current use of immunomodulating medication (including oral and inhaled steroids), self-reported symptoms of acute or recent infection (including use of antibiotics within last 3 months), taking lactulose or any other treatment for constipation, alcoholism and drug misuse. Additional exclusion criteria for older volunteers included: laboratory data which were outside the normal range for this age group and outside the ranges specified in the SENIEUR protocol [14], Barthel Index score of <16/100, cumulative illness rating scale (CIRS) score of >15 [15]. Additional exclusion criteria for the young subjects included laboratory data which were outside the normal range and influenza vaccination in the previous 12 months.

2.3. Sample size

The primary outcome of the trial was the antibody response to vaccination, incorporating mean antibody titres, vaccine-specific Ig subclasses and seroprotection and seroconversion. Power calculations were based on mean antibody titres. Since the influenza vaccine is trivalent, it is unlikely that an intervention will alter the response to all three subunits in the same way. For example, in the study of Davidson et al. [16], there was no effect of probiotic on mean antibody titres in response to the H1N1 subunit, whereas the responses to both H3N2 and the B subunit were improved (72 vs 51 [SD 16.5] for H3N2 and 31 vs 25 [SD 7.1] for B subunit). Based on the smaller effect size for the B subunit, a sample size of 26 subjects per group within each cohort was determined to be sufficient for a two-tailed significance level of 5% and a power of 80%; this was adjusted to 30 subjects per group to allow for dropouts. Data on the co-primary endpoints, immunoglobulin subclasses, seroprotection and seroconversion, is very sparse, but a sample size of 26 subjects per group within each cohort was determined to be sufficient for a 376 mg/dL difference in circulating IgG levels in response to influenza vaccination, with an SD of 438 mg/dL, a two-tailed significance level of 5% and a power of 80% [17]. A total of 62 young subjects and 63 older subjects entered the study and 58 young and 54 older subjects completed the study (Fig. 1). Two subjects experienced adverse effects (gastrointestinal bloating) during the study, one on the placebo group and one in the synbiotic group; both withdrew from the study.

2.4. Study design

Subjects consumed *B. longum bv. infantis* CCUG 52486 (*B. longum*, 10^9 CFU in 1 g skim milk powder/day) combined with gluco-oligosaccharide (Gl-OS (BioEcolians, Solabia); 8 g/day)in a double-blind, placebo controlled randomised parallel group study design for 8 weeks. The synbiotic approach was selected because *in vitro* data examining the growth and survival of this strain indicated that it was very vulnerable compared with other strains, but survived much better in the presence of an oligosaccharide substrate (data not shown). When comparing a number of possible substrates, the low water activity of Gl-OS, combined with its ability to support the growth of the probiotic strain, made it a clear choice for a powdered product. This prebiotic also has bifidogenic effects in batch culture models [18]. The placebo used was maltodextrin (9 g/day); both the placebo and the pre- and pro-biotic were sourced, packaged and blinded by BioAgro S.A. (Italy). The powders were consumed sprinkled into water or milk or with breakfast cereal. Microbiological safety of the product was

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