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Workshop Report

UK Food Standards Agency Workshop Report: Diet and Immune Function

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The UK Food Standards Agency convened a workshop on 13 May 2009 to discuss recently completed research on diet and immune function. The objective of the workshop was to review this research and to establish priorities for future research. Several of the trials presented at the workshop showed some effect of nutritional interventions (e.g. vitamin D, Zn, Se) on immune parameters. One trial found that increased fruit and vegetable intake may improve the antibody response to pneumococcal vaccination in older people. The workshop highlighted the need to further clarify the potential public health relevance of observed nutrition-related changes in immune function, e.g. susceptibility to infections and infectious morbidity.

Immune function: Diet: T lymphocytes: Cytokines: Vaccination: Selenium: Vitamin D: Zinc

The immune system is involved in host defence against infectious organisms and in assuring tolerance to sources of non-threatening antigens. Impaired immune function can be associated with increased susceptibility to infectious agents and increased severity of infections. Immune dysfunction and inflammation are now recognised to be involved in many disease states, e.g. CVD, cancer, atopic disease and obesity. There is increasing evidence that diet may have a direct effect on the immune system and consequently on disease risk. It is, therefore, important to understand the role of diet in modulating immune function, which, in turn, could provide an important functional outcome for formulating healthy eating advice. The UK Food Standards Agency (FSA) convened a workshop on 13 May 2009 at which the results from recently completed studies investigating diet and immune function, both FSA and non-FSA funded, were presented; the workshop was chaired by Dr Bernhard Watzl, Max Rubner-Institute. The FSA studies were commissioned in 2005 as a result of a review of FSA research, which highlighted the need for further work to investigate the role of diet on immune function[(1)].

Background

Professor Philip Calder, University of Southampton, gave an overview of immunological outcome measures and known dietary modulating factors. The immune system involves many different cell types, often with highly specialised functions; interactions among these are brought about by direct cell-to-cell contact and by the release of chemical mediators. The ability to combat infection is related to the ability to mount an effective immune response; however, the exact nature of the relationship is not well defined.

There are many laboratory measures of immune function and these show large between-individuals variation. Many factors, including genetics, sex, age, nutrient status and gut

Abbreviations: FSA, Food Standards Agency; NK, natural killer.

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flora, contribute to the observed variation. The 'clinical' relevance of variations in immune function measures between individuals is unclear[2]. Process for The Assessment of Scientific Support for Claims on Foods and the International Life Sciences Institute Europe Nutrition and Immunity Task Force have both addressed the use of immune markers in the context of nutrition and both concluded that trials should incorporate a comprehensive panel of immune markers[3,4].

Malnutrition and specific nutrient deficiencies (e.g. Zn deficiency) impair immune function and increase susceptibility to infection. Variations in intake and status of certain nutrients and dietary constituents (e.g. long-chain *n*-3 fatty acids, micronutrients, plant flavonoids, low-digestible oligosaccharides and probiotics) among free-living, fairly healthy individuals may account for some of the observed variations in immune function. The evidence for many dietary factors is, however, inconclusive, with some studies reporting effects and others not, e.g. the effect of fish oil supplementation on immune parameters in healthy subjects[5]; furthermore, the sensitivity of the immune system to dietary interventions may be different in subjects with an inflammatory condition compared with healthy subjects, for whom the immune system may be buffered to a larger extent against modulation by such intervention[5].

Nutritional modulation of immune function

Dr Jayne Woodside, Queen's University Belfast, presented results from a trial to determine the effect of increased fruit and vegetable consumption on immune function in the elderly. The immune system undergoes a range of changes, as individuals become elderly[6]. These may manifest as an increasing susceptibility to infection or a tendency to develop autoimmune or malignant disease. This study investigated multiple underlying factors contributing to immunological ageing, and the possibility that inadequate diet may be one such contributing factor. Fruit and vegetable intake, which can be low in the elderly, is associated with reduced chronic disease risk. This study tested the hypothesis that increased fruit and vegetable intake will positively affect clinically relevant measures of immune function.

Eighty-three healthy volunteers aged 65–85 years following a low fruit and vegetable diet ($\leq$2 portions/d) were recruited and randomised to continue following their normal diet or to consume at least five portions of fruit and vegetables daily for 16 weeks. At 12 weeks, tetanus toxoid and Pneumovax II were administered. Specific antibodies binding to tetanus toxoid (total IgG) and pneumococcal capsular polysaccharide (total IgG) were assessed by ELISA at baseline, 12 and 16 weeks. Natural killer (NK) cell cytotoxicity was assessed by flow cytometry at baseline, 12 and 16 weeks. Biochemical markers of nutritional status were assessed at baseline, 6, 12 and 16 weeks to monitor compliance.

Eighty-two participants completed the 16-week intervention. There were significant increases in plasma vitamin C, lutein and β-cryptoxanthin concentrations in the 5 portions/d group. There was no difference in antibody binding to tetanus toxoid between the two intervention groups, but antibody binding to pneumococcal capsular polysaccharide increased more in the 5 portions/d group than in the 2 portions/d group (geometric mean (95 % CI) change from baseline which represents percentage of baseline; 2 portions/d 179 (142, 227); 5 portions/d 299 (213, 421); P=0·02). NK cytotoxicity tended to be greater in the 5 portions/d group than in the 2 portions/d group at 16 weeks (P=0·07), and this effect was similar when cytotoxicity was adjusted for the proportion of NK cells (P = 0·07) but did not reach statistical significance. Overall, this trial suggests that increased fruit and vegetable intake may improve antibody response to vaccination in older people.

Dr Elizabeth Williams, University of Sheffield, presented the results from a trial to determine the effect of a dietary intervention on functional immune status in the elderly. Two hundred and seventeen older adults, aged 65–85 years, were recruited to a 3-month intervention trial with 3-month follow-up. Volunteers were randomised to one of the three treatment arms (dietary intervention, micronutrient supplement, placebo control) for the 3-month intervention period. The dietary intervention aimed to increase the intakes of Zn, Se, carotenoids, vitamins C and E. The micronutrient supplement contained micronutrient levels approximating the incremental increase expected to be achieved via the dietary intervention. Incidence of infection was recorded throughout the 6-month study period. Blood samples for the analysis of innate and adaptive immune function and nutritional status were collected at baseline, 3 and 6 months. Volunteers were vaccinated with tetanus vaccine after 8 weeks to determine the effect of the intervention on the responsiveness to a vaccine challenge.

The food intervention significantly increased plasma retinol, ascorbic acid, β-carotene, lutein, β-cryptoxanthin and Se concentrations. Plasma Se and vitamin C concentrations increased significantly in participants randomised to the micronutrient arm. There was no significant change in dietary intakes of the placebo and micronutrient groups.

At the end of intervention (month 3), NK cytotoxicity was significantly higher in the micronutrient arm compared with the placebo and the diet intervention arm (P=0·016 at the effector:target ratio of 12·5:1). There was, however, no significant difference in the change over time in NK cytotoxicity in any of the treatment groups. There was no significant difference in tetanus vaccine response between the treatment groups or in other measures of innate and adaptive immunity.

Self-reported proxy measures of infection over the 6-month duration of the study were significantly different across the treatment groups. Overall, increased intake of fruit and vegetables, fish and nuts was accompanied by an apparent benefit in self-reported symptoms and illness, which was not obviously explained by changes in innate or adaptive immune function.

Professor Susan Fairweather-Tait, University of East Anglia and Dr Kamal Ivory, Institute of Food Research, presented results from a trial on Se supplementation and immune function. A 12-week dietary intervention was undertaken in six groups (n 20) of adult men and women (aged 50–64 years) with low Se intakes Using a double-blind design, four of the groups were given daily capsules containing 50, 100 or 200 μg Se (Se-yeast, approximately 65 % selenomethionine) or placebo (control group), and the other two groups (double-blind) were given meals containing commercially available onions ($<$1 μg Se/onion) or Se-enriched onions (60 μg Se/onion approximately 66 % γ-glutamyl-methylselenocysteine) grown by Nottingham University. Each volunteer

in the two onion groups consumed three meals per week, which provided the equivalent of 50 μg Se/d (Se-enriched onions) or <1 μg Se/d (normal onions). The expression of Se-responsive genes and selenoproteins, enzyme activities and plasma Se concentration were determined over the 12-week period. The effects of Se status on the immune system were evaluated by studying cellular and humoural immune responses in individuals immunised at week 10 with influenza vaccine. Plasma selenoprotein P was found to be a more responsive biomarker of Se status than plasma Se, reaching a plateau after supplementation with 50 μg Se/d.

Se supplementation affected the immune parameters measured in various ways. A Se-induced enhancement of T cell proliferation was strongly correlated with both selenoprotein W gene expression and erythrocyte glutathione peroxidise-1 concentration. In general, Se-yeast appeared to have a greater effect on cytolytic cells, while Se-onion had more influence on their secretions. Overall, no single concentration of supplemented Se yielded a beneficial effect on all the immune parameters tested. Se supplementation did not affect influenza vaccine-induced Ig secretion. Although Se supplementation (100 μg/d) has been shown to improve several anti-polio vaccine immune responses[7], this trial, and others[8], have found no effect on influenza-induced Ig secretion, probably reflecting differences between the vaccines.

Professor Lothar Rink, RWTH-Aachen University, presented on Zn homeostasis and immunity. Zn is an essential trace element for the immune system and Zn deficiency compromises the immune function of all cells of the immune system. An excess of Zn, however, may have detrimental effects. Zn homeostasis, therefore, must be delicately regulated for an effective immune response. Recent years have brought a paradigm shift for the role of Zn in immunity. While its function as a structural component of many enzymes has been known for decades, current experimental evidence points to an additional function of the concentration of free or loosely bound Zn ions as an intracellular signal. The interaction of Zn with major signalling pathways that regulate immune cell activity and the implications of Zn deficiency or supplementation on Zn signalling as the molecular basis for an effect of Zn on immune cell function were presented[9]. In a trial in healthy elderly subjects, Zn supplementation (10 mg/d for 10 weeks) suppressed spontaneous release of pro-inflammatory cytokines, while increasing the Zn supplementation stimulated release of T-helper 1 cytokines, which are normally reduced during ageing[10]. Genetic background also affected the response[11]. A trial using higher doses of Zn (15 mg or 30 mg/d) observed no effect on immune function in healthy individuals[12]. While low-dose Zn supplementation may have beneficial effects, long-term high-dose supplementation does not and could even result in detrimental effects[9].

Dr Margherita Cantorna, Penn State University, presented on vitamin D and the immune system, in relation to autoimmune disease prevalence. Emerging evidence suggests that low vitamin D status may be associated with an increased risk of autoimmune diseases like multiple sclerosis and inflammatory bowel disease[13]. Mice that are either vitamin D deficient or vitamin D receptor deficient (KO mice) are prone to develop autoimmunity. There are paradoxical effects of vitamin D on immunity[14]. Experiments in animals support a suppressive effect of vitamin D on autoimmunity, but not other aspects of immunity including host resistance to infectious disease and asthma. Current data suggest that vitamin D and the vitamin D receptor are required for the development and function of at least two regulatory populations of T cells, invariant NKT cells, that are early produces of cytokines and CD4/CD8αα intraepithelial lymphocytes found in the gastrointestinal tract[15]. Protective immune responses that depend on these regulatory T cells are therefore impaired in the absence of vitamin D or the vitamin D receptor leading to an increase in autoimmunity of the gut and other tissues. These studies were performed in laboratory animals and information is currently lacking as to the effect of vitamin D interventions on the immune system in human subjects. Based on results from animal studies, it has been hypothesised that decreased vitamin D status and/or dietary intakes of vitamin D may be associated with the severity and/or prevalence of multiple sclerosis, asthma and some infections (e.g. tuberculosis)[16]. More evidence is needed in human subjects, however, to determine if there is a casual link between changes in vitamin D, regulation of the immune system and disease outcome.

Discussion

Several of the trials presented at the workshop show some effects of nutritional interventions on immune parameters. For example, increased fruit and vegetable intake may improve antibody response to pneumococcal vaccination in older people and, also in older people, Zn homeostasis was shown to modulate cytokine release. It will be important to follow this work up by determining how this relates to susceptibility to infections and infectious morbidity, as affecting the activity of one or more components of the immune system may not necessarily be of any benefit (or harm) to the individual[2].

There are many laboratory measures of immune function and these show large inter-individual variation. Individuals with immune responses significantly below 'normal' are more susceptible to infectious agents and exhibit increased infectious morbidity and mortality; however, it is unclear how the variation in immune function among healthy individuals relates to variation in susceptibility to infection[2]. When selecting populations for diet and immune function studies, it may be appropriate to screen and select the subjects based on response to a given parameter or assay, e.g. NK cell cytotoxicity.

As highlighted in the ILSI report, technical factors need to be standardised to minimise variability in immune parameters and assays[3]. In the case of response to vaccination, seasonality in viral exposure or seasonal variation in diet and nutritional status will need to be considered; while choosing older people as subjects may be more relevant, but any comorbidities will also need to be described and quantified.

Research recommendations

(1) Human trials investigating vitamin D and immune function, although several trials are ongoing.
(2) Dietary interventions of health relevance and immune function, including further clarification of the potential public health relevance of observed nutrition-related changes in immune function.

Participants

Dr Berhnard Watzl, Professor Philip Calder, Dr Margherita Cantorna, Professor Susan Fairweather-Tait, Dr Kamal Ivory, Professor Lothar Rink, Dr Liz Williams, Dr Jayne Woodside, Dr David Edgar, Dr Rachel Hurst, Professor Malcolm Jackson, Dr Frank McArdle, Professor Graham Pockley, Dr Parveen Yaqoob, Dr Peter Sanderson, Dr Alison Tedstone, Dr Elaine Stone, Rachel Elsom, Verity Kirkpatrick, Heiko Stolte, Anne Milne, Sarah Hardy, Elizabeth Kendall.

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