Evaluating next generation ingredients to support immune health

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What is the immune system?

**Innate**
- Physical barriers i.e. skin
- Natural killer cells
- Macrophages

**Acquired**
- Cell-mediated
  - T and B cells
- Humoral
  - Antibody mediated
Balance is vital

Immune function too low – greater risk of infection

Just right

Immune function too high – greater risk of autoimmune and allergic disease
Influenced by our gut bacteria and diet

Diet influences the composition and metabolic capacity of commensal bacteria.

Bidirectional relationships

Markers of immunity

**Blood**
- Prostaglandins
- Natural killer cells
- Interleukins
- T-cell subtypes
- Cytokines e.g. INF-\(\mu\), TNF

**Gut**
- Microbiota profiling & quantification
## What we measure and why

<table>
<thead>
<tr>
<th>Marker</th>
<th>What it shows</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostaglandins</strong></td>
<td>Lipids produced in response to infection and inflammation</td>
</tr>
<tr>
<td><strong>Natural killer cells</strong></td>
<td>Types of lymphocyte that stimulate cytotoxic T cells and contribute to cell death</td>
</tr>
<tr>
<td><strong>T ‘helper’ cells</strong></td>
<td>Type of lymphocyte that is central to adaptive immunity</td>
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<tr>
<td><strong>Cytokines</strong></td>
<td>These are produced by immune cells and include interleukins &amp; tumour necrosis factor</td>
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<tr>
<td><strong>Interleukins</strong></td>
<td>Proteins which act on various components of the immune system e.g. activating T cells, &amp; macrophages</td>
</tr>
<tr>
<td><strong>Tumour necrosis factor</strong></td>
<td>A cytokine able to trigger apoptotic cell death, destroying pathogens</td>
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</tbody>
</table>
Gold standard biomarkers

• International Life Sciences Institute - European branch

• Key organisation – working on the best assessment methods & means of interpreting immune markers

Source: Calder et al. (2014) *Endocr Metab Immune Disor Drug Targets* [Epub ahead of print].
Life stages

Stage 1 – *in utero* programming/infancy

Stage 2 – *Modulating* normal immune function in *infants*

Stage 3 – *Modulating* normal immune function *later in life*

Stage 4 – *Boosting* immune function
STAGE 1: PROGRAMMING
Maternal supplies vital

The timing of immune dysregulation in the brain has been linked to neurodevelopment disorders e.g. autism spectrum disorder.

The developing immune system is sensitive to internal & external signals.

Maternal & fetal immune systems communicate in a bidirectional manner.

The maternal immune systems tolerate foetal antigens.

Deficiencies of Zn, vits A, D, E, choline, B vits and folate have clear roles in cell-mediated and immune responses.

All of these have implications for later immune function.

Source: Marques et al. (2013) Front Neurosci 7: 120.
Omega-3s in pregnancy

- n-3 fatty acids anti-inflammatory
- n-6 fatty acids pro-inflammatory and linked with allergic disease
- Pregnancy fish oil consumption - found to reduce sensitisation to food allergens and risk of atopic dermatitis in offspring during the first year of life

Study: fish oil RCT

• N=83 atopic pregnant women given fish oil (3.7g PUFA/d) from 20 wks gestation until delivery (Australia)

• In supplemented group:
  – Infant PUFA status better and cytokine responses lower
  – Lower risk of food allergy and less severe dermatitis in sufferers

Study: fish oil

• N=1094 pregnant women given fish oil (0.4g DHA/d) from 18 wks gestation until delivery (Mexico)

• In supplemented group who had atopic mothers:
  – Significantly less nasal congestion and fever up to 18 months post-natally

STAGE 2: MODULATION IN THE EARLY YEARS
Gut colonization in early life

- The gut harbours 500-1000 microbial species
- Central to gut & systemic immune function
- Colonization in early life impacts on immunity throughout life

The first 1000 days of life is when the body is trained to respond to external stimuli.

Gut microbial development is achieved at around 3yrs of age.

Types of microflora

Harmful:
- diarrhea/constipation infections systemic effects
- prodn. of potential carcinogens
- prodn. of toxic H₂S
- intestinal putrefaction

Beneficial:
- inhibit growth of exogenous and/or harmful bacteria (competitive exclusion, antimicrobials, low pH)
- stimulate immune functions through non-pathogenic means, anti-tumor properties, cholesterol reduction
- lower gas distension
- aid in digestion and/or absorption of food ingredients/minerals synthesis of vitamins

Pathogenic (incl. production of toxins):
- Ps. aeruginose
- vibriocaceae
- staphylococci
- clostridia

Veillonella
Enterobacteria
E. coli
Lactobacilli
Sulphate reducers
Anaerobic G⁺ cocci
methanogens
eubacteria
Bifidobacteria
bacteroides

http://members.shaw.ca/duncancrow/fig4.GIF
Supplementation studies
Study: fish oil

- Double-blind RCT in infants
- Received fish oil (280mg DHA, 110mg EPA) vs. control from birth to 6 months
- Allergies assessed at 6 and 12 months

n=420 infants at high risk of atopy; blood samples for n=120

Results

- DHA and EPA levels sig. higher in fish oil group
- Infants with higher DHA levels had lower Th2-type T cells (i.e. less allergenic response)
- Conclusion: n-3 PUFA may protect against allergies in future

Study: fish oil

- Randomised 2x2 factorial design study (cow’s milk vs. formula with/without fish oil)
- Dose of fish oil equivalent to a teaspoon per day from 9 to 12 months of age
- Whole blood samples cultured and stimulated with *L. paracasei* from infants (challenge test)
- Immune response measured

Conclusion: fish oil leads to faster immune maturation

Study: probiotics

• Double-blind RCT

• Probiotics or placebo given 1 month before delivery to months & 6 months to infants with a history of allergy

• The probiotic group had sig. higher CRP and total IgA, IgE & IL-10

Results

Increased plasma CRP was also associated with ↓ risk of eczema & allergic disease at 2yrs

Study: pre- vs probiotics RCT

• N=94 preterm infants given probiotic \((Lactobacillus)\) vs. prebiotic (oligosaccharide) vs. placebo for 2 months

• Significantly lower risk of respiratory tract infection seen in both supplemented groups vs. placebo

• Less rhinovirus-induced episodes in prebiotic group vs. placebo

STAGE 3: MODULATION IN LATER LIFE
EFSA Health Claims

Folate
Vitamin A
Vitamin B12
Vitamin B6
Vitamin C
Vitamin D
Iron
Zinc
Selenium
Copper

“Contribute to the normal function of the immune system”.

Source: http://ec.europa.eu/nuhclaims/
Folate

• Hereditary folate malabsorption has been linked to immunodeficiency

• Possible impaired T cell response
  – Similar effects for folate deficiency?


4-7% females <LRNI in UK NDNS
Vitamin A

- Deficiency impairs innate immunity
  - Regeneration of mucosal barriers is impaired
  - Function of neutrophils, macrophages and natural killer cells is reduced
  - Plays a role in T cell and B cell function

Vitamin D

- Cod liver oil used in past to treat TB
- Immunodulatory and anti-cancer effects
- Vit D enzymes and receptors are present in immune cells e.g. T and B cells and monocytes

20% teens and adults clinically deficient

Sources: Prietl (2013) Nutrients 5(7): 2502-21
Vitamin E

• Certain nutrients may help to prevent immunosenescence
  – The decline of the immune system with age

• Vit. E may help to preserve T cell function in older people

Wu et al. (2014) Endocr Metab Immune Disord Drug Targets[Epub].
Zinc

• Zinc acts as an intracellular signal molecule for immune cells

• Zinc supplementation has been found to reduce levels of inflammatory cytokines in elderly patients with sickle cell disease


12-22% teenagers and 7% adults <LRNI
Iron

- In response to infection, the innate immune system preserves iron leaving blood stores low
- Fe deficiency linked with higher risk infection


46% girls and 23% of women <LRNI
Selenium

- Se supplementation lowers levels of Th2-type helper cells (can trigger allergic responses) and boosts Th2-type helper cells (believed to help protect against cancer and viruses)


26% men and 51% women <LRNI
Probiotics – no official claims

Most studied probiotics in relation to immunomodulatory effects.

- *Lactobacillus rhamnosus GG*
- *Bifidobacterium animalis Bb-12*
- *Lactobacillus casei Shirota*
- *Lactobacillus johnsonii La1*
- *Bifidobacterium lactis DR10*
- *Saccharomyces cerevisiae boulardii*

Proposed actions of probiotics

• Thought to enhance:
  – Activation of macrophages
  – Natural killer cells
  – T-lymphocytes
  – Cytokine release
  – Breast milk IgA
  – Gut defences via increased % of ‘good’ bacteria

STAGE 4: BOOSTING FUNCTION
Issues with studies

- Baseline nutritional status
- Dose and duration of supplementation or dietary change
- Compliance
- Interpretation of complex results
- Few studies have health outcome data e.g. % infections
Zinc

- ZENITH Study, healthy subjects 55-70yrs
  - Supplementation with 15 or 30mg Zn daily for 6 months
  - Total Zn intake up to 40 mg daily (diet + supp.) had no significant long-term impact on immune status
  - Poor compliance, low dose or normal baseline zinc status?

Zinc + β-carotene

- Interleukin-6 production was 16% higher with zinc supp.

- Interferon-γ production was 36% lower with β-carotene supp.

Double-blind RCT, women supplemented during pregnancy then 136 infants followed for 6m

Zinc for the Common Cold

- 16 therapeutic and 2 preventative trials (n=394)
- Zinc intakes >75g/d* associated with a sig. reduction in the duration of days but not the severity of cold symptoms


*RDA = 10mg
Similar positive finding for vit. C

- Review using systematic approaches

- Vit. C (500mg-1000mg) may **reduce the duration of cold symptoms** in adults and children but does not reduce the severity of symptoms

Study: vitamin D

- 4-month double blind RCT in 20 post-menopausal women
- Randomised to one of two forms of vit D
- Both types of vitamin D (20 µg/day) contributed to a decrease in five out of seven markers of innate immunity


Vitamin D RDA = 5µg
**Iron + vitamin A**

- 2-month double blind RCT n=186 anaemic pregnant ⚗

- Randomised to take:
  - 1) 60mg ferrous sulfate or 2) 60mg iron + 0.4mg folic acid or 3) 60mg iron, 2mg retinol + 0.4mg folic acid

- **Iron+retinol** was most effective at improving lymphocyte proliferation than iron alone

Conclusions

- Zinc, vit. C & E
- Omega-3 fatty acids
- Vit. D
- B vitamins
- Iron
- Bioactives
- Probiotics & prebiotics

Good  Moderate  Emerging
• Nutrient intakes & deficiencies can affect the immune system
• Intakes of immune nutrients can be low – especially **selenium, vitamin D, iron**
• Health claims “contributes to the normal function of the immune system” have been approved for 10 nutrients
• Need more research on gold standard markers and how to interpret these
Practical applications

- **Pregnancy** – fish oil supplementation
- **Infanthood** – fish oil, vitamin D, pre- or probiotics may be useful
- **Adulthood** – ensure that vitamins and minerals meet recommended levels and consider a supplement if required
- **Illness** – vitamin C and zinc helpful for colds, probiotics could help stomach upsets
Acknowledgment

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