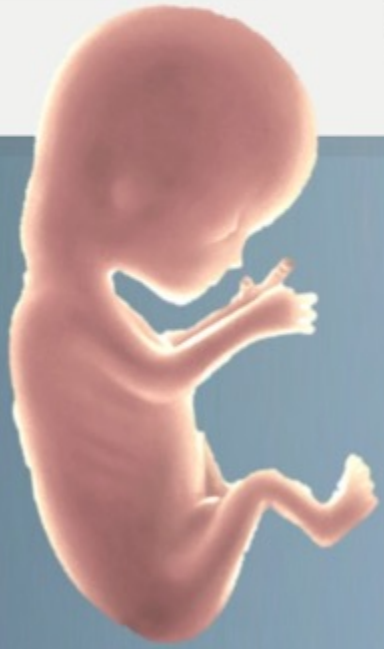
WUN
Worldwide Universities Network

INTERNATIONAL INFLAMMATION NETWORK

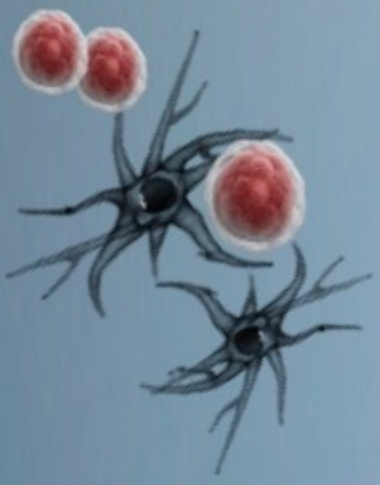
in-FLAME

THE UNIVERSITY OF WESTERN AUSTRALIA



The International inflammation Network

5th Annual Workshop and 2016 REPORT



Maastricht, Netherlands,
April 1-3, 2016

"Early life strategies to reduce inflammation for long-term health"

Maastricht University

cost

Universiteitsfonds Limburg
1965 - 2015 | 50 JAAR SWOL

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in-FLAME

WORLDWIDE UNIVERSITIES NETWORK

Introduction from our *in-FLAME* Network Chair:

Prof Susan Prescott, University of Western Australia, Telethon Kids Institute

Another resounding success! Maastricht provided a beautiful setting for our largest, most productive and enjoyable meeting yet. The University and surrounds provided the perfect historic backdrop to build stronger friendships and robust science and we welcomed many new network members. It was good to see the strong collaborative spirit of in-FLAME more alive than ever. As always, the main goal of this workshop was to create a collaborative space for creative discussions, productive partnerships and long term relationships. Continuing the themes of our previous meetings, this workshop had a core focus on key early exposures - namely nutrition, the microbiome, early microbial diversity, nature relatedness, pollutants and the built environment - and how these interact to modify early immune development, to impact many aspects of development. Our ongoing multisystem focus includes a range of early outcomes including allergy and asthma, obesity and metabolism, mental health and behaviour. It was an honour to have the **WUN Executive Director John Hearn** launch our meeting. Now entering our 5th year, the *in-FLAME* has continued to mature, and has quickly become a showcase for collaborative success within the WUN global challenge program.

As ever, we are indebted to **Prof Diane Campbell** (University of Sydney) and **Prof Anita Kozyrskyj** (University of Alberta) for their help in all of our ongoing network activities. This year we had the wonderful opportunity to work with local organisers **A/Prof Carel Thijs, Dr. John Penders** and **Dr. Monique Mommers** on behalf of Maastricht University. Their major contribution to the program, fundraising, the venue and social activities of the meeting made for a stimulating, seamless and enjoyable meeting for all - from the opening event in the wine cellar of Thiessen, to our night adventures walking in the Marl Caves before dinner at Slavante, the perfect backdrop for our packed scientific discussion.

Prof Dianne Campbell and Prof Susan Prescott

For the first time we had **a Systems Biology Workshop**, led by local expert **Prof Ilja Arts** (Maastricht Centre for Systems Biology, MaCSBio). We also our second BIRTH LactoActive Workshop. We also also proud of the active participation of so many early career researchers who have been working together to create their own network, led by **Dr Daniel Munblit** (Imperial College London) with help from **Dr Chrysanthi Skevaki** (Marburg), and with support from **Prof Anita Kozyrsky** (University of Alberta). Going forward, we plan to focus not only on our research collaborations, but also our communications and engagement, including in the public domain – recognising that advocacy is as important as science in promoting a healthier future for all.

I am looking forward to welcoming you to our next workshop in **New York, May 3-5, 2017**.

Prof Susan Prescott
Founding Chair, *in-FLAME* Network
http://www.wuninflame.org.

A warm welcome from our Maastricht hosts

Following an inspiring meeting in Marburg last year, it was our pleasure to welcome in-FLAME to our home in Maastricht in 2016! We welcomed the opportunity to provide both a stimulating scientific and social program, with ample informal opportunities to exchange ideas with colleagues, find new collaborations and new inspirations. We hope that all enjoyed the pleasant atmosphere of this old provincial town, now home of the youngest Dutch university. We are extremely grateful to our local sponsors **Maastricht University, University Fund Limburg, Brightlands Health Campus**, who made this meeting possible.

A little bit more about our beautiful city, especially for those who could not join us: Maastricht is located in the most southern part of the Netherlands on both sides of the river Meuse. It developed from a Roman settlement, to a religious centre, a garrison city and an industrial city. Nowadays, it is known as a city of history, culture and education. Maastricht University, founded in 1976, celebrates its 40th anniversary this year. After Amsterdam, Maastricht has the highest amount (1677) of national heritage sites in the Netherlands. The city has become internationally known, by way of the Maastricht Treaty, as the birthplace of the European Union, European citizenship and the single European currency.

We hope that the delegates all enjoyed the atmosphere of the wonderful cellars at Thiessen Wijnkoopers where we had our opening session and Keynote presentations (photographed below), and the excursions to the famous Marl Caves followed by Dinner at Slavante.

It has truly been our pleasure to host this meeting and we look forward to many more to come.

John Penders, Monique Mommers, Carel Thijs
WUN *in-FLAME* local organising committee, Maastricht University

About the *in-FLAME* Network

Launched in 2012, the *in-FLAME* Network addresses the risk factors, pathways and strategies to overcome the rising propensity for chronic inflammatory disorders, with a focus on early effects on the developing immune system. Led from UWA by Professor Susan Prescott, it involves 9 WUN universities and WUN+ partners from >50 institutions, and >25 countries around the world. Now with over 200 current members, we are working on an integrated program of population studies, biological studies and intervention studies aimed at preventing inflammation and the burden of subsequent disease.

'*…There has been an unprecedented rise in non-communicable diseases (NCDs) such as allergies, asthma, cancer, diabetes, mental ill health and obesity. Inflammation and immune dysregulation are common features, often associated with similar environmental and lifestyle risk factors such as dietary patterns, environmental pollutants, microbial patterns and stress. Given the central role of the immune system in health and development, inflammation must be examined as both a common element and target for the prevention of NCDs…*'

For further information please visit our website or the WUN general links:
http://www.wuninflame.org.
http://wun.ac.uk/wun/research/view/in-flame-international-inflammation-network
http://wun.ac.uk/article/early-life-solutions-to-the-modern-health-crisis

Our alignment with WUN goals and values

WUN's values underpin our approach to maximising the network's core strengths.

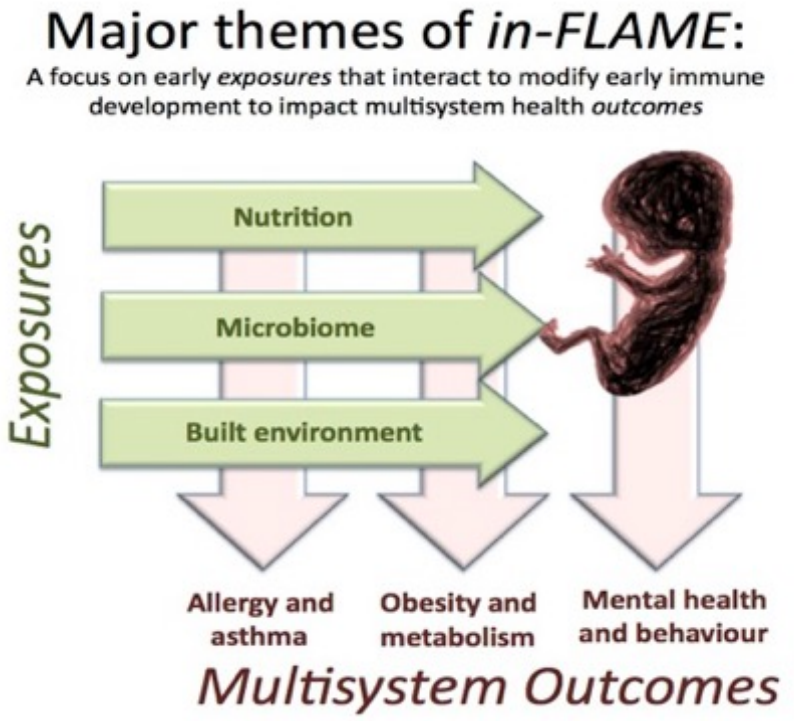
DEVELOP	NURTURE	INNOVATE	COLLABORATE	IMPACT
WUN actively develops our global research alliance and education programs to meet our objectives.	WUN nurtures and invests resources in the intellectual strengths and priorities of our members.	WUN seeks and instigates innovation in knowledge management and educational exchange.	WUN promotes opportunities that accelerate internationalisation for global collaborations between universities and with government, international organisations and industry on issues of global significance.	WUN exists to make a difference. Our research produces new knowledge that influences policy and impacts society to improve lives.

OBJECTIVES & STRATEGIES

1	Strengthen and grow our university network	• Develop and maintain a dynamic portfolio of research and education programs in alignment with WUN members' priorities. • Strategically grow the membership as a network of peer universities with mutual strengths and regional diversity. • Build ownership and leadership within WUN to increase collaboration, commitment and sense of community.
2	Foster influential research communities	• Focus our efforts on four *Global Challenges*: – Responding to Climate Change (food security, urbanisation, oceanography); – Public Health (lifecourse approaches to obesity, heart disease, diabetes); – Global Higher Education and Research (access and equity, new technologies); – Understanding Cultures (migration, digital futures, ageing). • Incorporate cross-cutting themes in big data, macroeconomics, and regional programs on China and Africa into our *Global Challenge* programs. • Strengthen leadership and accountability to ensure quality and delivery.
3	Nurture research talent	• Create opportunities for the engagement and career development of talented researchers at the postdoctoral, postgraduate and undergraduate level in international research collaborations. • Facilitate the mobility of students and academic staff. • Promote equity for researchers in our programs.
4	Enhance the WUN profile	• Position WUN as a recognised thought leader in our areas of expertise, engaging the WUN Presidents and experts as an international think-tank and as policy advisors. • Increase the power of the "WUN voice" in an ambassadorial and lobbying role. • Strengthen the WUN brand and profile with internal and external audiences, ensuring WUN is recognised for the vast potential of its intellectual resources.

Goals of the *in-FLAME* 2016 workshop

A new and overarching goal of this workshop was to develop our profile and new projects around the **impact of dysbiotic drift on global health**, as a common unifying link to many aspects of health and environmental exposures. Given the multisystem impact of the environment through immune health, particularly in early life, the in-FLAME group of experts are well positioned in this area. **This provides an integrative systems framework for understanding the eco-biological impact of living environments (including biodiversity) on microbial diversity and life-course human health from the perspective of omics and nature relatedness.** In addition our ongoing goals, under this broader banner, were to develop and consolidate our research platform through new and existing projects – in ways that may be connected through the. Continuing the themes of our previous meetings, we have a core focus on key early exposures (namely a) **nutrition**, b) the **microbiome** and early microbial diversity, and c) **pollutants** and the built environment), and how these interact to modify early immune development, to impact many aspects of development. Our multisystem focus includes a range of early outcomes including 1) **allergy** and asthma 2) **obesity** and metabolism 3) **mental health** of behaviour – as previously outlined in out network map.



Travel Grant recipients:

Thanks to generous support from Danone Nutricia and our academic sponsors we were very pleased to provide travel grants for early and mid career researchers, to attend the meeting and present their work. Congratulations to:

Maresa Botha
Kate mcCloskey
Kristina Rüter
Samantha Dawson
Siobhain M O'Mahony
Ayşe Kılıç
Majda Dzidic
Laurence Macia
Fiona Collier
Catherine Li Lai
Manjeet Kumari
Siddhartha Mandal



Meeting sponsors:

The meeting was generously supported by a UWA Research Collaborative Award (RCA), **Maastricht University, University Fund Limburg, Brightlands, Danone, and COST (European Cooperation in Science and Technology).** We are particularly grateful to **Prof John Holloway** and **Prof Susanne Kraus-Etschmann** for facilitating this a COST partnership. We are also grateful to our WUN collaborators from the University of Sydney and University of Alberta and all the in-kind contributions of participating members.

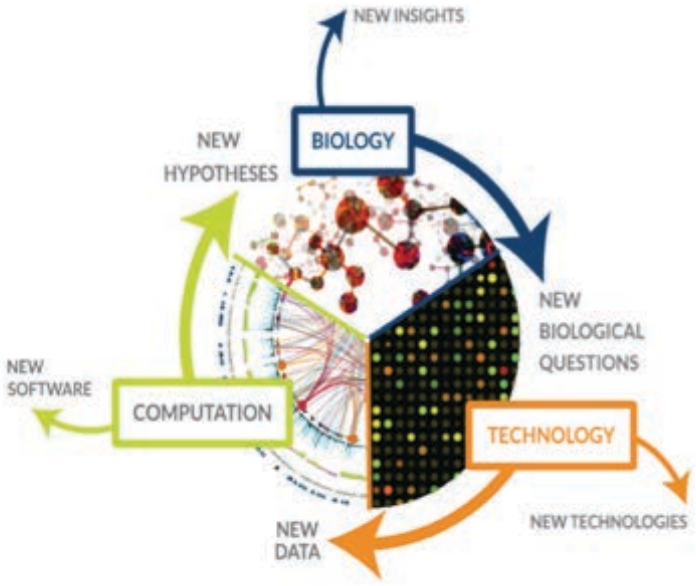

Report: Systems Biology Workshop

Prof. Ilja Arts
Dr John Penders
Maastricht University

macsbio
MAASTRICHT CENTRE FOR SYSTEMS BIOLOGY

A new 2016 in-FLAME initiative

For the first time this year we held a Systems Biology pre-workshop in collaboration with the recently established Maastricht Centre for Systems Biology (MaCsBio). The main goal of this workshop was to discuss the different methodological approaches within the field of Systems Biology and their potential applications in studies using (multi)-omics data in association to early immune development and non-communicable diseases. In the introductory lecture by **Prof Ilja Arts**, director of MacsBio, provided insight in the value of systems biology in studying complex diseases by integrating multi-omics data with information on environmental/life-style aspects. Using the example of metabolic syndrome she pointed out the importance of deep phenotyping in diseases with a heterogeneous aetiology and disease course. Furthermore, she highlighted the different modelling approaches being applied within the field of systems biology: data driven analysis, mathematical modelling and pathway analysis. The subsequent invited speakers, each addressed of these approaches in more detail.



Computational modeling of human gut microbes

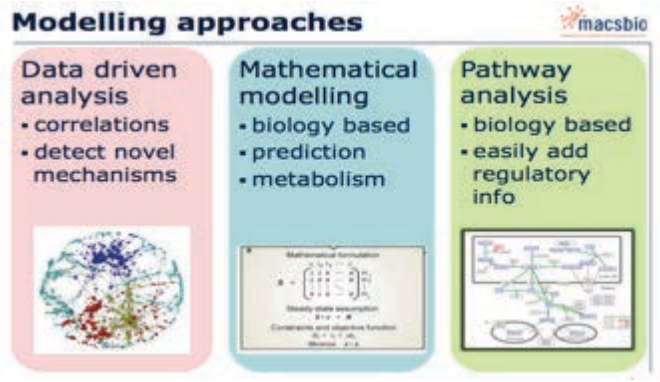
A/Prof. Ines Thiele (Luxemburg)

Computational modeling of metabolism has gained increasing attention for biotechnological and biomedical applications. Such modeling is achieved by assembling in a bottom-up manner a high-fidelity computational representation of an organism's metabolic network based on genomic, biochemical, and physiological data. Various computational tools exist to either characterize its phenotypic properties (e.g., amino acid or vitamin production capabilities). A next step is to expand this metabolic modeling approach to microbial communities. Ines introduced newly developed tools, which enable modeling of microbial communities. She presented their semi-automated assembly pipeline for the assembly of high-fidelity microbial metabolic networks for microbes inhabiting the human gut (anaerobes and aerobes). The application of this pipeline was illustrated to generate a collection of >300 gut microbial metabolic networks, amenable to metabolic modeling, which were also phenotypically characterized in silico. Next, a computational modeling toolbox for microbial communities permitting the simulation of large-scale microbial community models as well as the tailoring of the microbial community models based on meta-omics data was outlined.

Complexity and reductionism in the omics era

Prof. Kristel van Steen (Liege, Belgium)

Kristel van Steen nicely illustrated the use of data reduction in complex –omics datasets and highlighting the two ends of the spectra from a reductionist point of view (molecular biology) to a holistic point of view (systems biology).
Different data-reduction methods were described as well as the challenges including replication, validation and interpretation of findings were highlighted. Also the inclusion of environmental data or omics data related to other organisms (e.g. microbiome, virome) by using propensity scores were outlined, including the challenges related to dynamic exposures and



missing data and confounding factors. Everything being put into perspective by the alternative approaches from a holistic point of view, Kristel concluded that combining the strengths of the reductionist and holistic view are the proper way to go in the omics era.

Pathway analyses & nutrigenomics

Prof. Chris Evelo (Maastricht, The Netherlands)

Pathway analysis are the third form of modelling approaches being used in the field of systems biology and **Chris Evelo** highlighted the use of pathway analysis and nutrigenomics. Chris introduced wikipathways, an open collaborative platform for capturing and dissiminating models of biological pathways for data visualization and analysis. Standalone tools such as PathVisio and Cytoscape can be used to support the pathway analysis and visualizations. Some nice illustrations were shown and the extensions of wikipathways by including other data (e.g. miRNA's) or specifically visualize pathways in specific organs was discussed. (www.wikipathways.org)

Following the presentations by our guest speakers, the workshop continued by presentations by members of our own network.

Studying the infant microbiome: COPSAC2010

Dr**. Jakob Stokholm** (Copenhagen, Denmark) shared his data on the infant microbiome analysis in COPSAC2010. 16S sequencing was performed on a total of ~1700 samples collected at the ages of 1 week, 1 month and 1 year. With increasing age, microbial

diversity increased and the microbiota composition became more mature

(less facultative anaerobic species and bifidobacteria, increase in many other genera, including Bacteroides, Prevotella and butyrate-producing clostridial clusters) with the samples collected at age 1 year clustering apart from the 1 week and 1 month samples. Additionally, Jakob highlighted the application of PICRUST to infer the functional capacity by imputing the metagenome from 16S sequencing data, illustrated

by the example of birth mode as a determinant for the metabolic capacity at the age of 1 week.

A Systems Biology approach to the gut microbiome

A/Prof **Susanne Brix** (DTU, Denmark) illustrated the widely unannotated gut metagenome and the methods that can be applied to still identify microbial species with specific functional properties without the availability of reference genomes.The most recent published gene catalogue for gut metagenomes contains a total of almost 10 million genes, but as little as ~12% of these genes can be found back in the currently available reference genomes. Until recently, de novo segregation of complex metagenomic data into bacterial species/strains remained problematic. However, binning genes based upon their co-abundance across a series of metagenomic samples, has been shown to enable the assembly of microbial genomes (MGS or "metagenomic species") without the need for reference genomes. Over 70% of the MGS are to date unknown species, with an overrepresentation of unknown MGS within the Firmicutes phylum. Susanne moreover showed that data from the LPS biosynthetic pathway indicated that most gut Gram-negative species carry the non-inflammatory penta-acylated LPS variant.

Novel methods to study longitudinal microbiota patterns and infant growth

Dr. **Siddharta Mandal** (New Dehli, India) presented the statistical framework called ANalysis of Composition of Microbiomes (ANCOM)*. The method accounts for the underlying structure of the data and can be used for comparing the composition of microbiomes in two or more populations. The method does not make any distributional assumptions and can be implemented in a linear framework enabling the adjustment for covariables or modelling of longitudinal data. Using the data of the Yatsunenko paper on the microbiomes of subjects from the USA, Malawi and Venezuela and focussing on the infants in this dataset, ANCOM could clearly identify differences in many OTUs between the infants from the USA on one hand and the infants from Malawi and Venezuela on the other hand.

*software available at: "http://www.niehs.nih.gov/research/atniehs/labs/bb/staff/peddada/"

Summary

This first systems biology workshop has been a great success, demonstrating the different tools and approaches that are being available and are already or can be applied in studies integrating –omics and environmental data in association to NCDs, ranging from data reduction methods, mathematical modelling tools and pathway analysis.

Especially in the field of microbiome analysis many methods are becoming available, such as methods to infer functional capacity based on 16S data, methods for de novo assembly of microbial genes into metagenomic species, differentiation between inflammatory/non-inflammatory Gram-negatives and statistical methods that enable correction for confounding variables and longitudinal data.

We want to thank everyone who attended the pre-workshop 'Systems Biology' meeting.

Report: in-FLAME 'LactoActive' Workshop

Prof Anita Kozyrskyj
University of Alberta

Dr Daniel Munblit
Imperial College London

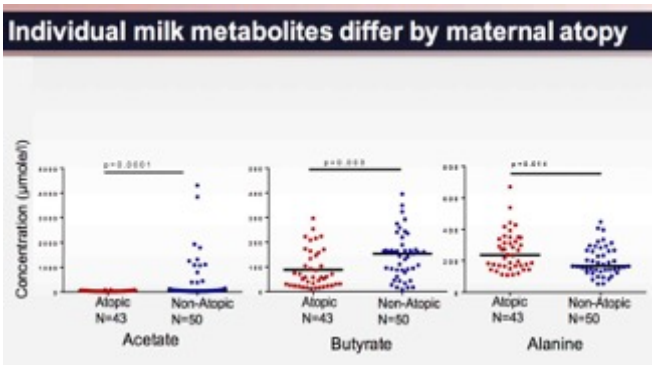
For the second year, our breast milk interest group head, Lactation Biome-Active Partners Against NCDs (LactoActive), held a pre-workshop meeting to discuss our projects in collaborations with 'Symbiota' and other groups.

Breast milk metabolites cluster by maternal atopy

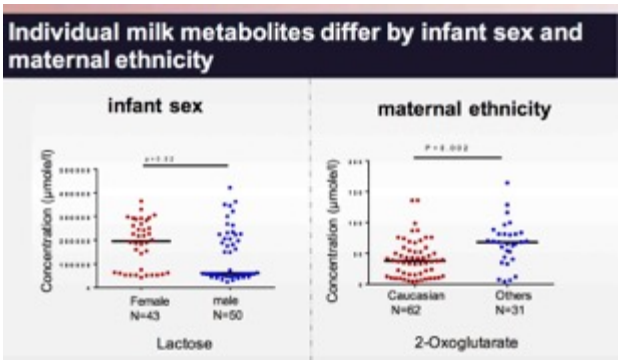
Professor **Anita Kozyrskyj** and postdoctoral fellow **Manjeet Kumari** opened the meeting by sharing data from the multicenter collaborative project, comparing samples from Norway, Japan, South Africa Australia and North America. This is comparing key metabolites of the breastmilk metabolome in relation to maternal atopic status, infant sex, geographical location and maternal ethnicity.

The study is employing Nuclear Magnetic Resonance (NMR) as a rapid non-destructive method of identification and quantification of metabolites. Samples had profiles from 51 metabolites belonging to Sugar metabolism, fatty acid metabolism, primary metabolism and amino acid metabolism. Principal component analysis revealed clustering by geographical location. There was also clustering for maternal allergy status, which may have reflected differences in atopic predisposition between regions.

There were also differences based on infants sex and maternal ethnicity. A number of specific breastmilk metabolites different according to maternal atopy, including acetate, butyrate and alanine.



Lower abundance of SCFA & intermediates in breast milk from atopic mothers could reflect differences in maternal microbio and have functional implications. This is consistent with previous studies reported Lower SCFA synthesis by breast milk microbiota, with fewer bifidobacteria in breast milk from atopic mothers at 1 month, and correspondingly fewer bifidobacteria in the gut microbiota of their newborns (Gronlund 2007). Others have also reported lower SCFA synthesis by gut microbiota and transfer to milk link to allergic outcomes - with higher gut aerobes (Enterobacteria) and lower serum acetate in mid to late pregnancy associated with increased risk of infant wheeze (Lange 2012, Thorburn 2015). In a murine



model low SCFA levels, acetate & propionate, predicted allergic phenotypes (Thorburn 2015, Trompette 2014). Finally, lower gut iso-butyrate levels in children at age 1 have been linked with allergic sensitization at age 4 (Sandin 2008).

SUMMARY OF KEY FINDINGS

1. The Country specific clustering of breast milk metabolites observed was attributed to atopic status of mother
2. Variations according to maternal ethnicity and infant sex were also seen
3. Reduced abundance of SCFAs in breast milk metabolites could be the link to elevated risk of atopy and NCDs in offspring of atopic mothers

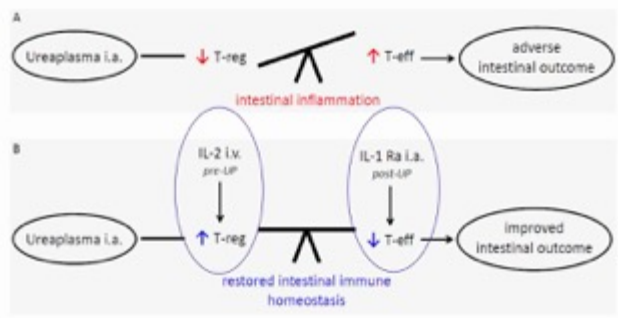
Breast milk fatty acids and health outcomes in children

Lenie van Rossem presented the outcomes in the PIAMA Birth Cohort: Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort, in relation to breast milk fatty acids. PIAMA is a cohort of 3963 Healthy newborns in 1996/1997 in the Netherlands. Follow-up has been by annual questionnaires, and medical examinations. In this study 661 Eligible mothers were asked to collect breast milk; no restrictions to time, pumping method, or feeding phase. 276 successful samples were collected. Samples were put in a tube where oxidation was prevented and send by mail and stored at -70° C. Fatty acids were analyzed with gas liquid chromatography. These measures were examined in association with a number of health outcomes. A relatively high content of N-3 LC PUFAs – in particular DHA – in breast milk were associated with lower blood pressure, less asthma, and better school performance in healthy children.

Antenatal immune modulation and postnatal gut pathologies; important lessons for feeding strategies

Tim Wolfs discussed the implications of pre-term birth on neonatal gut development, including at this outcomes such as perforation, poor feeding and growth, and necrotising enterocolitis (NEC) which may be associated with compromise gut barrier function and innate immune defence. chorioamnionitis is the most important cause of preterm birth (bacterial infection of the amniotic fluid, placenta and membranes). Regardless of premature labor chorioamnionitis is an independent risk factor for intestinal pathologies such as NEC. However, mechanisms underlying the association between chorioamnionitis and the increased incidence of postnatal gut pathologies are unknown.

He presented an ovine chorioamnionitis model, based on injections of micro-organisms or their components in the amniotic fluid at different gestational ages. This has advantages over other animal models, including longer gestational period, as in humans. Better potential for surgical interventions and administration of pharmacological interventions. This provides a number of opportunities to understand the underlying Mechanisms of the association between chorioamnionitis and the increased incidence of postnatal gut pathologies. I have used this model to investigate the effects of infection or contamination of the amniotic fluid with microbes (ureaplasma) or microbial products (LPS). These interventions were associated with IL-1 mediated inflammation of the fetal gut with altered T-reg/T-eff ratios. There were



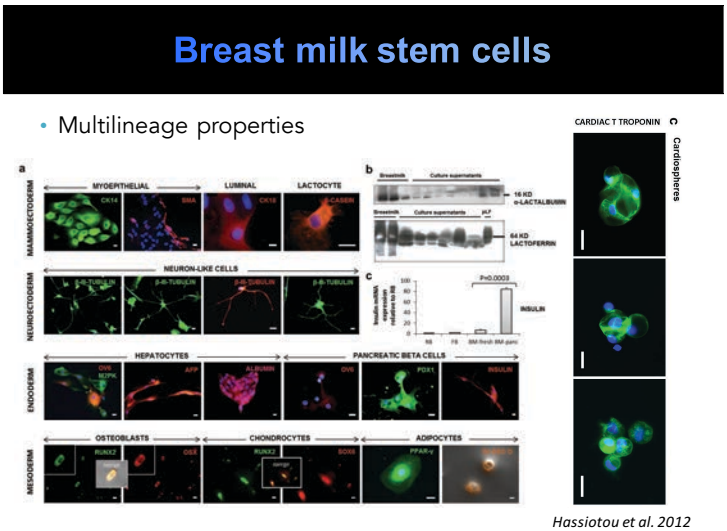
also associated effects on epithelial cells, with damage to villus enterocytes and disturbs programming of enterocytes (KLF5). This suggests that and tonight or factors may be involved in the adverse outcomes in the neonatal bout, and offers antenatal opportunities for both treatment and prevention through immunomodulation - including maternal nutrition and other factors which may influence the microbiome.

Breast milk molecules, metabolites and cells: research from Down Under

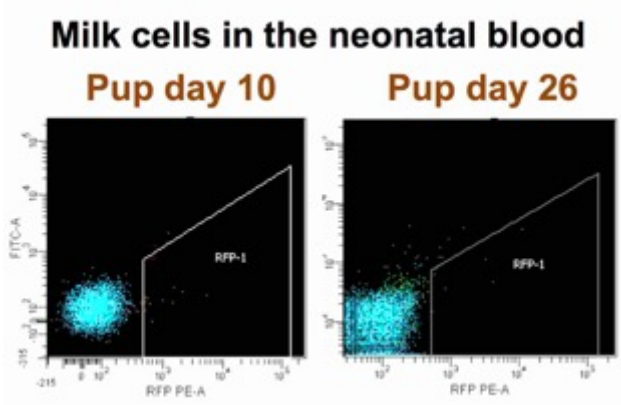
Donna Geddes gave a comprehensive summary of the many aspects of breast milk research being undertaken at the University of Western Australia. The general goals of her team at to build a more extensive knowledge
base of milk synthesis, composition, milk removal, milk storage and infant feeding. They are examining various aspects of breastmilk supply and composition in relation to both short and long term health outcomes of breastfed infants. For example, she gave results of the recent longitudinal study of feeding volume which revealed that infant percentage fat mass positively associated with 24 hour feed volume (p=0.033). She also outline specific studies on fat levels in human milk, including comparison of Gravimetric, Creamatocrit and Esterified fatty acid (EFA) methods for the determination of total lipid content in human milk. Comparing that content with these three different measures has revealed that both Creamatocrit and EFA had good correlations with Gravimetric method. Smaller average errors were obtained for Creamatcorit method than that for EFA method. Creamatocrit method could be used as an alternative to the reference Gravimetric method. Another advantageous comes from the fact that Crematocrit is simple and employs very small samples. She also outline specific studies looking at trace elements (Zinc Copper Iron in particular) and different pasteurization methods for use in human milk banks. Metabolomics is another major In this area their major aims are:

- To characterize components in milk and develop a comprehensive database.
- To understand mammary gland physiology and metabolism.
- As a diagnostic approach (identification of women at high risk for mastitis).
- To understand what is being transferred from mother to child.

Perhaps the area of greatest fascination to the audience was the role of **breast milk stem cells.** Donna outlined evidence that multi-lineage stem cells present breast milk survive the infant stomach and can be detected in neonatal blood. Animal models show how milk stem cells reach the liver to differentiate into functional hepatocytes. Milk stem cells can also be detected in the pancreas and the spleen. In the thymus, milk stem cells differentiate into thymocytes. Finally, milk stem cells have also been detected in the brain, differentiating into neurons (*Hassiotou et al. 2014*). These fascinating studies provide evidence of migration and functional integration of native milk stem cells to organs of the neonate. This highlights how arranged of factors which influence immune and stem cell composition (such as infections or antibiotics) could have implications for many aspects of infant development.



Hassiotou et al. 2012



Our proposed interactions with the European Milk Bank Association (EMBA) – representation by our ECR network

Daniel Munblit (Imperial College) indicated that he will be travelling to Milan in May to the European Milk Bank Association meeting - representing in-FLAME ECR group. This will be an opportunity to raise particular issues during my discussions with EMBA board. Based on discussions during our Maastricht meeting and subsequently with **Donna Geddes (UWA)** the main aims of the ECR endeavors will be:

1. To provide reference ranges for immunological components of HM and its changes over time in different geographical locations
2. To provide evidence on HM microbiome in different geographic locations
3. To study protein and energy content in different geographic locations
4. To examine HM immunological composition for distinct patterns using principal component analysis and/or latent cluster analysis
5. To assess main maternal and environmental factors having impact on HM immune composition
6. To find an association between HM composition and infant health outcomes (if we manage to seek enough financial support for this part of the project)

Addendum: Following the in-FLAME Meeting, Daniel reported a successful meeting with EMBA in Milan, with the first steps towards a successful collaboration. This included discussions with Gillian Weaver, a former EMBA president and leader of EMBA guidelines group, who is very much in support of our desire to collaborate. The following points were discussed (as summarized by Daniel):

Although there are some variations between breast milk collection in different countries, many things consistent and may form the basis of a multicenter collaborative initiative, including consent taken from the mothers in many milk banks cross Europe.

- All breast milk donors undergo serological testing which allow potential serum/BM markers paired comparison. There are some studies on this topic but they are limited in numbers.
- All mothers are screened for smoking, drugs, infection. Women are also asked if they are vegetarians and if they take B12 supplements. We discussed a possibility of having a supplementary dietary questionnaire for the donors and it seems a reachable goal.
- Age of the baby for the women to stop donating breast milk varies from 6 months up to 12 months in some countries. This definitely falls into the range most of us interested in.
- In regards to method of expression – most milk banks do not provide specific encouragement for hand expression, but all accept pump-expressed milk; pump should be sterilized), with no particular guidance on the breast nipple area treatment before extraction.
- There could be issues with regards to "type" of milk expressed (hindmilk/foremilk, full breast) as it would be difficult to control. We need to think how to solve this issue in the best way.
- In some milk banks freshly expressed milk is added to already frozen milk but most countries do not have this practice.
- Breast milk is discarded if it does not meet particular criteria (e.g. culturing positivity), I am wondering if some of these samples could be used for methodological research.
- Most of the banks use very rigorous sample labeling with name of donor, date of collection/expression, donor's number, identification of bank, date of freezing, batch number, date to be transported to central breast milk bank, medications taken by donor recorded. Fresh milk goes to the refrigerator straight after collection in

many countries.

- Worthwhile noting that raw milk is generally stored for 6-12 months in the freezer before pasteurization.
- Freezing conditions for collected samples vary, with usual requirement of samples to be stored at a temperature not warmer than – 20C°. Milk banks accept breast milk storage in a variety of containers which is worth noting considering their subsequent analysis for potential research purposes. It may affect some analysis but not the others.
- It seems that guidance around the Europe is to minimize exposure to sunlight and/or phototherapy lights (this seem to be the case in most of the countries). Many banks allow pooling of pre-pasteurized milk from the same donor (this is true for most countries); with mixed approach regarding pooling breast milk from different donors. We need to keep it in mind for future discussions but I don't see it a major problem as we will be most probably getting a particular volume of pre-frozen samples aliquoted.
- First donation of breast milk from the donor undergoes bacteriological testing in most of the countries, milk is also screened for total viable microorganisms. Verification of Dornic acidity, content/creamatocrit is undertaken. Type of treatment (primarily Holder pasteurization) recorded.
- Post pasteurization tests include microbiological count for all countries.

It is very interesting to note that in some countries (Norway, Austria, parts of Germany) raw donor breast milk is used (whilst the vast majority of countries around the globe pasteurize their donated milk). This opens possibilities for a comparative research in this field considering decades of raw milk use in some countries.

Daniel and Donna Geddes have also started drafting a brief grant proposal to Larsson-Rosenquist foundation. I draft will be circulated in the coming weeks after discussion with EMBA, to potentially incorporate their views and suggestions.

Summary and outcomes:

Subsequent to discussions at the LactoActive workshop, a letter of expression was submitted to the JPI (Joint Transnational Research Proposals on "Biomarkers for Nutrition and Health") funding opportunity on April 19, 2014 to add breast milk samples from the Dutch KOALA cohort and the MecMilk cohorts from Italy, Russia and the UK. The proposed research will link breast milk metabolite levels to atopic disease outcomes in adolescents. Partners on the proposed research include Canada (Kozyrskyj), Netherlands (Thijs), Italy (Peroni) and the UK (Boyle and Munblit), with a dollar commitment from Nutricia. Norway (Eggesbo) and Australia (Geddes) are also collaborators on the proposal, as well as the LactoActive network. Finally, on their way to the University of Alberta Metabolomics Centre for NMR processing are additional breast milk samples from the US WHEALS birth cohort and from a sample of non-atopic women living in Perth obtained by Donna Geddes.





Relationship between milk microbiota, bacterial load, macronutrients and human cells during lactation*

Alba Boix, Maria del Carmen Collado, Alex Mira

Human breast milk is considered the optimal source of nutrition for healthy infants, providing essential nutrients and a broad range of bioactive, immunological and protective compounds unmatched by formula feeding, as well as its own microbiota. However, the interaction among those bioactive compounds and the biological role of milk microorganisms is still uncovered. Thus, our aim was to identify the relationships between milk microbiota composition, bacterial load, macronutrients and human cells during lactation. Bacterial load was estimated in milk samples from a total of 21 mothers through lactation time by specific qPCR targeted to total bacteria. Milk microbiome composition and diversity was estimated by 16S-pyrosequencing and the structure of these bacteria in the fluid was studied by flow cytometry, qPCR and microscopy. Fat, protein, lactose and dry extract of milk content as well as the number of somatic cells were also analyzed.

We observed that milk bacterial communities were generally complex, and showed individual-specific profiles. Milk microbiota was dominated by *Staphylococcus, Pseudomonas, Streptococcus* and *Acinetobacter*. There was no correlation between bacterial load and the amount of immune cells in milk, strengthening the idea that milk bacteria are not sensed as an infection by the immune system. Overall, the data support that milk microorganisms are symbiotic and their potentially beneficial role should be elucidated.

KEY FINDINGS

The median bacterial load was around 10^6 bacterial cells/ml through time, a 100-fold higher concentration than previously estimated by culture.

Milk bacteria were not found only present in a free-living, "planktonic" state, but also in equal proportion associated to human immune cells, which could imply that bacteria could reach the mammary gland travelling adhered to these cells through the blood stream or lymph.

Breast milk TGF-B2 is associated with neonatal gut microbiome composition

Alexandra Sitarik, Suzanne Havstad, Kevin Bobbitt, Ganesa Wegienka, Christine Johnson (*Detroit, Michigan, USA)*

Though breastfeeding is one of the most important determinants of neonatal gut microbiome composition, breast milk is a complex bioactive fluid with its composition depending on a multitude of maternal and environmental conditions. One dynamic breast milk component that regulates inflammation—potentially by inducing changes in microbiota—are cytokines, with the TGFβ family being the most abundant. We hypothesized that variations in breast milk composition, measured by cytokines, further explains bacterial variation among breastfed neonates. The study population consisted of maternal-child pairs enrolled in the Wayne County Health Environment Allergy and Asthma Longitudinal Study (WHEALS), a racially and socioeconomically diverse birth cohort based in Detroit, Michigan. The analytic sample consisted of participants who reported breastfeeding at the 1-month study visit and had a neonatal stool and breast milk sample collected and analyzed at this time (N=52). Gut microbiome was profiled using 16S rRNA sequencing, and breast milk TGFβ1, TGFβ2, and IL-10 were assayed using the Luminex multiplex platform. Compositional differences in the microbiome were evaluated using permutational multivariate analysis of variance with the Unweighted UniFrac distance matrix. Tests of differential taxa abundance were performed using zero-inflated negative binomial regression with false discovery rate adjustment (0.01 significance threshold). In conclusion, breast milk TGFβ2 further explains bacterial variability in the gut of breastfed neonates. Whether TGFβ2 acts in isolation or jointly with other bioactive components to alter bacterial composition requires further investigation.

KEY FINDINGS

We found that after adjusting for maternal race, breast milk TGFβ2 was significantly associated with neonatal gut microbial composition, but TGFβ1 and IL-10 were not ($p=0.043$, $p=0.25$, $p=0.81$, respectively). Individual taxa tests adjusted for race revealed that higher levels of TGFβ2 were associated with increased abundance of several *Bifidobacteriaceae*, *Streptococcaceae*, and *Ruminococcaceae* taxa. These findings may contribute to a further understanding of how breastfeeding affects immune development in early life.

Mechanisms of early life priming for allergy by house dust mite allergen in breast milk: role of protease and impact of prebiotics

Akila Rekima, Chrystelle Bonnart, Astrid Hogenkamp, Nathalie Vergnolles, Johan Garssen and Valérie Verhasselt

There is a need to identify protective and risk factors for development of allergies to slow down allergy epidemic. We have shown in a mouse model that house dust mite allergens in maternal milk are responsible for antigen specific increased risk for respiratory allergies and for break of tolerance to unrelated dietary antigen present in breast milk and increased risk for food allergy. We aimed to investigate whether protease activity of HDM allergens is responsible for its pro-allergenic properties in breast milk and whether maternal administration of non digestible oligosaccharides can counteract deleterious effect of HDM in milk. *L*actating mothers were exposed by respiratory route to HDM extract, HDM extract with neutralized protease activity or to HDM extract plus a diet supplemented in non digestible oligosaccharides. Offspring were tested in young adulthood for susceptibility to allergy.

KEY FINDINGS

We found that neutralization of HDM protease activity totally abolished its allergic priming activity in the offspring. Mechanisms of action of HDM protease will be described at the in-Flame meeting as well as impact of non digestible oligosaccharides administration to mothers. Protease activity of HDM is critical for its allergy priming activities upon transfer through breast milk. Modulation of maternal milk capacity to inhibit HDM protease activity should be an important target to investigate in human cohorts to ensure prevention of allergy by breastfeeding

Impact of colostrum, the first physiological food, on early post-natal and adult metabolic and immune homeostasis

Akila Rekima, Johan Garssen and **Valérie Verhasselt**

Colostrum, the very first physiological food, differs profoundly qualitatively and quantitatively from mature breast milk. Colostrum is poorly investigated for its health benefits. This is key since there is a widespread lack of colostrum administration to neonates which may increase susceptibility to allergic and metabolic disease risk. We will study in mice the impact of colostrum on the early development of immune system and early life metabolic homeostasis. We will further assess the consequences of lack of colostrum intake on obesity and allergic diseases in adulthood. We will analyze the impact of selected colostrum factors, i.e. Vitamin A, oligosaccharides, colostrum-induced microbiota, and their mechanisms of action on these parameters.

KEY FINDINGS

Our preliminary results indicate that a lack in colostrum administration results in major defect in weight intake during breastfeeding period, delayed immune system development and long term increased susceptibility to allergy. This project will lead to the identification of dietary factors and physiological actors in early life which condition long term health and allow proposing preventive nutrition for allergy and obesity

The effect of maternal dietary egg intake in early lactation on human milk ovalbumin concentration: a randomized controlled trial

Jessica Metcalfe, Julie Marsh, Nina D'Vaz, Donna Geddes, Ching Tat Lai, Susan Prescott, **Debra Palmer**

Limited evidence exists about how maternal diet affects breast milk food allergen concentration, or whether infant exposure to allergens in breast milk influences the development of oral tolerance or sensitisation. The aim of this study was to investigate the effect of maternal dietary egg ingestion during early lactation on the amount of egg protein (ovalbumin) detected in human breast milk. In a randomized controlled trial, women were allocated a dietary group for the first six weeks of lactation: high egg diet (> 4 eggs per week), low egg diet (1-3 eggs per week) or an egg free diet. Breast milk samples were collected at two, four and six weeks of lactation for the measurement of egg protein (ovalbumin). Egg-specific IgE and IgG4 were measured in infant plasma at six weeks, and prior to the introduction of egg in solids at 16 weeks. At six and 16 weeks maternal and infant transepidermal water loss (TEWL) were measured. Maternal egg ingestion is associated with breast milk ovalbumin content, and tolerance markers in infants. These results highlight the potential for maternal diet to benefit infant oral tolerance development during lactation.

KEY FINDINGS

A maternal diet of at least four eggs per week resulted in higher ovalbumin concentrations in breast milk than an egg free diet ($p=0.03$). Average maternal egg ingestion was also associated with breast milk ovalbumin concentration, where ingesting an additional one egg per week resulted in an average 25% increase in ovalbumin concentration. Infant egg-specific IgG4 levels were positively associated with maternal average egg ingestion ($P=0.02$), with an increase in one egg per week resulting in an average 22% increase in egg-specific IgG4 levels. Maternal and infant TEWL readings correlated at both six ($P=0.002$) and 16 weeks ($P<0.0001$).

We want to thank everyone who attended the pre-workshop 'Lacto-Active' meeting. Please contact Anita Kozyrsky kozyrsky@ualberta.ca if you would like more information or want to add new aspects to these collaborations.

Main Meeting
New Friends and New Ideas:

Once again we welcomed many new collaborators to the ***in-FLAME*** network this year, now with over 250 members across more than 50 institutions. A major focus of our meeting this year was to develop unifying concepts that bring together the multisystem effects of broad-ranging environmental influences early in life. Many of these - including modern nutritional practices, contaminants and pollutants, stress, declining environmental and microbial biodiversity, sedentary behavior, and our growing disconnect from nature - have effects on the developing immune system and are driving the increasing predisposition to inflammation, metabolic dysregulation and the rising noncommunicable diseases (NCDs).

This year we had a major focus on the microbiome, and both the causes and the consequences of declining biodiversity and modern 'dysbiotic drift'. Our three keynote speakers (**Karsten Kristiansen**, **Alan C. Logan** and **John Penders**) where selected to address this from different perspectives, with a view to integrating our approaches to these challenges. This included combining a broad ecological view of global health disparities and environmental issues (biodiversity loss) with a deeper systems biology approach to understanding course for pathways and mechanisms. Over the course of the meeting list this led to a overarching **in-FLAME Global Environment Working Group** to provide an integrative systems framework for understanding the eco-biological impact of living environments (including biodiversity) on microbial diversity and life-course human health from the perspective of omics and nature relatedness (as out-lined on Page 28). We see this initiative as a critical overarching perspective which integrates all of the working activities within our network, while providing some focused themes for collaborative projects, such as applying 'nature relatedness' scores to existing cohorts, and assessing this in relation to measures of microbial biodiversity, metabolic signatures (and other 'omics technologies), and measures of health and well-being. It was agreed that our network is uniquely placed in this arena, and is one of the few groups taking this broad perspective. As we develop our footprint in this area we anticipate a range of new funding and collaborative opportunities. A major outcome of the meeting will be to develop and publish a 'perspective' paper that will clearly outline the vision and contribution of the in-FLAME network in this regard.

Keynote presentation 1:
The Gut Microbiota in Health and Disease

Karsten Kristiansen, **Professor of Molecular Biology Head, Genomics and Molecular Biomedicine, University of Copenhagen,** Senior advisor to BGI-Shenzhen, China

The importance of the gut microbiota for regulation of metabolism and immune functions is well established, and evidence has been presented that the gut microbiota may also affect behavior. However, the exact molecular mechanisms by which bacteria in the gut exert their actions still remain elusive. Kristiansen's laboratory is involved in large-scale metagenomics projects in collaboration with BGI-Shenzhen using high throughput Illumina-based sequencing of total fecal DNA. These studies have primarily been focused on humans and mice, but have now been extended to encompass several other species including pigs. In his lecture he summarized data on the mouse, the pig, and the human gut microbiomes, pointing to differences and similarities. In relation to studies on humans, their projects are in particular focused on characterizing changes the gut microbiota associated with metabolic disorders, cancers and autoimmune diseases. In studies of human cohorts they have recently described changes in the gut microbiome that characterize obese individuals, individuals with type 2 diabetes, and patients suffering from rheumatoid arthritis and colorectal cancer revealing characteristic changes in the diversity and functional competences of the gut microbiota. He also summarized recent results demonstrating how metagenomics analyses can be used for early non-invasive diagnosis of colorectal cancer, describe examples of how such analyses can be used to predict efficacy of treatment in relation to rheumatoid arthritis, and even stratify type 2 diabetic patients prior to start of treatments. He concluded the lecture by discussing possible functional consequences and perspectives of these findings.

Keynote presentation 2:
The Microbe-Mind Connection and Global Dysbiosis. Why the Big Picture Matters

Alan C. Logan

Independent researcher and Invited faculty, Harvard's School Mind-Body Medicine Continuing Medical Education since 2005 Interest in the influence of natural environments, microbiota, biodiversity and their collective role in personal, community, and global health. He set forth the first contemporary framework for the ways in which the manipulation of microbiota could influence fatigue (2003) and depression (2005).

The idea that microbes are part of an immense ecosystem service - one that contribute to human health, and ultimately the well-being of the Earth itself - seems undeniable. Recently, we and others have begun to ask if there is a 'microbiota of modernity', and if so, how might modern pressures influence human-associated microbiota? Although the term dysbiosis has morphed into a term centered largely around alterations in gut (and other anatomically-located) microbes, its original definition is quite simple: ***life in distress***; ***difficult living***. Given the realities of climate change and biodiversity loss, it is easy to argue that planetary life is in distress. Therefore dysbiosis is a global issue. Furthermore, scientific advances have made it reasonable to ask if modern changes in human behavior and lifestyle (including diminished contact with biodiversity, of which microbes are a part) may also be causing 'difficult living' for humans.

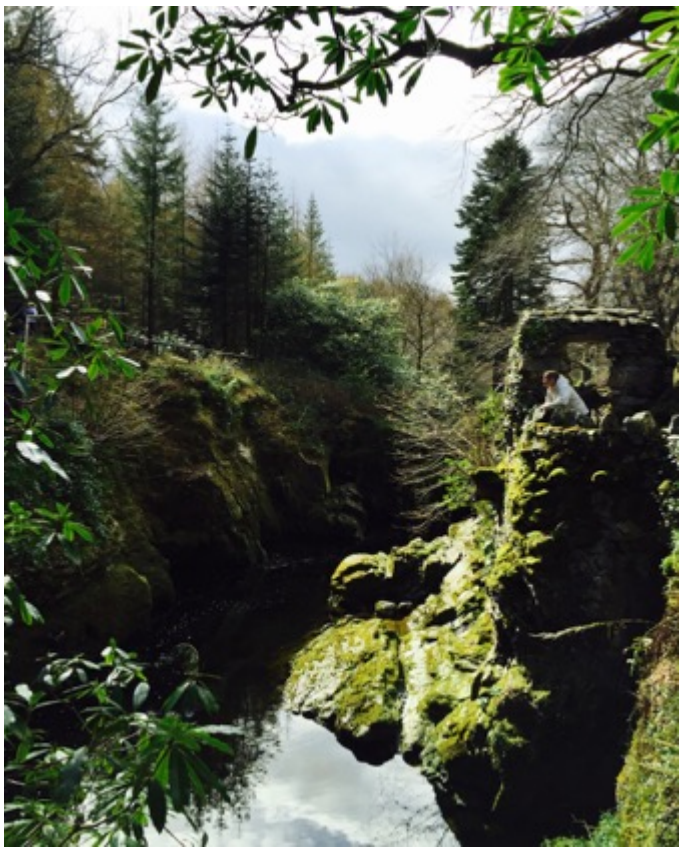
The modern pressures that might influence microbiota include:
1. Rapid urbanization (e.g. aspects of the built environment, cognitive demands, stress)
2. Westernization of dietary patterns (e.g. mass introduction of ultra-processed foods)
3. Lifestyle (e.g. sedentary, less time spent in natural environments)
4. Widely applied pharmaceutical applications (e.g. antibiotic use)
5. Climate factors (e.g. thermal stress, biodiversity loss)

Accumulating evidence suggests that urbanization and westernization are factors which contribute to dysbiosis in its more recognizable, microbial-related definition. Here, dysbiosis refers to perturbations to the structure of complex commensal microbial communities. More specifically, it involves the loss of beneficial microorganisms, and/or the expansion of potentially harmful microbes, and/or the loss of overall microbial diversity. Groups maintaining very traditional non-urbanized and non-westernized lifestyles, are generally differentiated by far greater alpha-diversity of faecal microbiota.

These differences in microbial diversity are not merely of interest for intellectual fancy. Although these more isolated groups - arguably living in ways that more closely resemble our ancestors - may be more susceptible to mortality early in life, they experience non-communicable diseases (NCDs) much less frequently throughout life. Since there are now volumes of research demonstrating that microbial dysbiosis can have a detrimental effects on local and systemic physiology (e.g. lipid and glucose regulation), it seems imperative to learn more about the ways in which microbiota of modernity might be mediators of health and disease.

Microbial dysbiosis is, of course, a relative term. It is likely that there are degrees of differences in microbial diversity among truly isolated communities and those that might be in transition. In addition to what we might learn from isolated, non-westernized groups, it is also likely that the most disadvantaged in developed countries, those that shoulder the largest NCD burden, will display a state of dysbiosis. We refer to this as dysbiotic drift. The environmental forces contributing to such a drift - beyond the obvious dietary factors - are discussed here. Studies in North America and Brazil support the notion that dysbiosis can sit along a socioeconomic gradient in developed and transitioning countries.

Physiological anthropology (PA) is a unique hub for many specialized branches of science and medicine. PA seeks to understand the *physiological* consequences of modern pressures placed upon humans, most notably those that might differ from our ancestral past. Fundamentally, these physiological consequences may be part of a broad evolutionary mismatch that contributes to a global epidemic of NCDs. Just as surely as PA is a science with a rich multi-disciplinary interest, so too is the 'microbiome revolution' a great unifying factor in science and medicine. Microbiota (formerly known as 'microflora' or 'flora') residing on and within humans, as well as their collective genomes, are referred to as the microbiome. PA and the modern microbiota is highly relevant to our collaborative group, the International Inflammation (in-FLAME) Network of the Worldwide Universities Network (WUN); we are interested in the ways in which the physiological consequences of individual and synergistic modern pressures might contribute to non-communicable diseases (NCDs) via immune mechanisms. Over time, if these pressures are not met with adaptation, intervention, resilience and/or appropriate coping, a destructive tandem of chronic low-grade inflammation and oxidative stress can damage cells - like waves eroding a shoreline. Virtually all NCDs - from allergic diseases to schizophrenia - have been associated with an inflammatory burden.



However, PA and groups such as in-FLAME are not focused solely on NCDs. There is also a need to understand the role of the microbiota-immune interface as it relates to general health. As the World Health Organization correctly points out, health is not merely the absence of disease. Mental health is not the absence of a DSM-V or ICD-10 diagnosis - it is quality of life and the ability to reach one's potential.

The symptoms of subsyndromal/subthreshold mental disorders, chronic fatigue, gastrointestinal complaints and circadian disruptions are exceedingly common in modern environments. They are associated with low-grade inflammation (e.g. elevated inflammatory cytokines) and they also place one on a trajectory toward formal NCD diagnoses. Therefore, the immune system, which has always collaborated with microbiota in health and disease throughout the evolutionary history of our Genus, has found itself center stage in discussions of microbiota and health. For the previous two decades this was primarily confined to research in allergic diseases and asthma, but no more.

It is entirely possible that insights gained from the study of microbes as an ecosystem service - most especially when the effects of urbanization/westernization are reflected off traditional-living populations - will pay off in untold ways. Of course we have much to learn and much collaborative work to be completed before any promissory notes are cashed. However, the concentrated microbiome research is *already* forcing hard questions concerning human behavior, environmental forces pushing dysbiosis (by all of its definitions) and global health.


THE BLOG

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David Suzuki Foundation

Creating a sustainable Canada through research, education and policy

Connecting With Nature Has Real Health Benefits

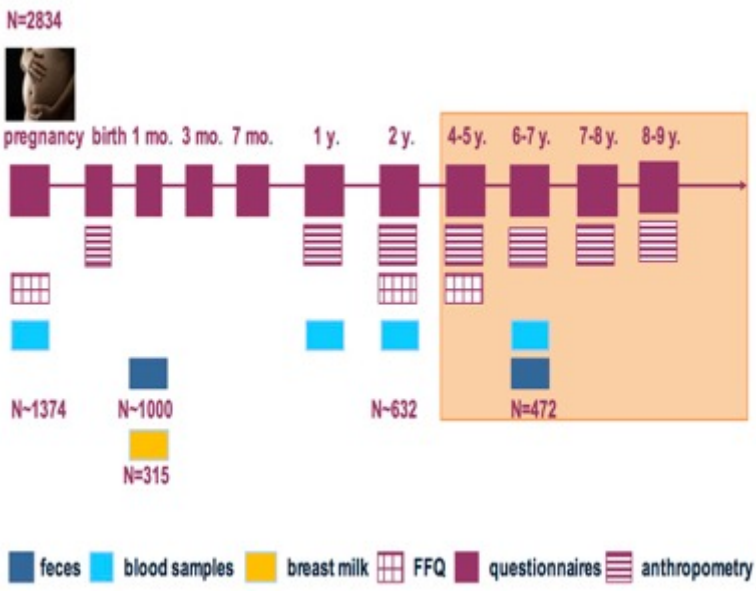
By Susan L. Prescott and Alan C. Logan

Our first meeting was on a bus in Tokyo, Japan, in 2003. We began talking about the influence of mindfulness and natural environments on human health. Microbes are an unseen part of nature and an essential part of the biodiversity that sustains life. Thus, we wondered about the relationship between biophilia and the hygiene hypothesis.

Keynote presentation 3:
First results of metagenomics analysis from KOALA

John Penders
Dept. of Medical Microbiology
Maastricht UMC+

As a leading expert in microbiome analysis and as one of our key hosts for the meeting it was our pleasure to invite John to give a keynote presentation updating us on the KOALA cohort. The main focus of his talk was the relationship between the developing microbio and metabolism, And the factors which may influence this, Including early antibiotic usage. In his introduction he reviewed the still limited human studies linking the microbiome with obesity, highlighting how some studies have reported an association of ⇓ level of Bacteriodetes with obesity *(Ley et al. 2006, Armougom et al. 2009, Turnbaugh et al. 2009, Zuo et al. 2011)* while others have found the opposite (*Balamurugan et al. 2010, Duncan et al. 2008, Mai et al. 2009, Karlsson et al. 2012, Bervoets et al. 2013*). Some studies have also found associations between specific bacterial species and obesity (e.g. Lactobacillus spp, E. coli, and bifidobacteria). Studies in children remain limited and most of these have been done using traditional culture based or PCR techniques. Furthermore, most have been case-control studies. The main aim of John and his collaborators has been to assess the developing faecal microbio of school age children ((7.5±0.8 yrs) in relation to overweight and metabolic derangements, using samples from the KOALA study – an ongoing population-based prospective birth cohort study (n=2834) in the Netherlands (*Kummeling et al: Pediatr Allergy Immunol 2005;16(8):679-48*).



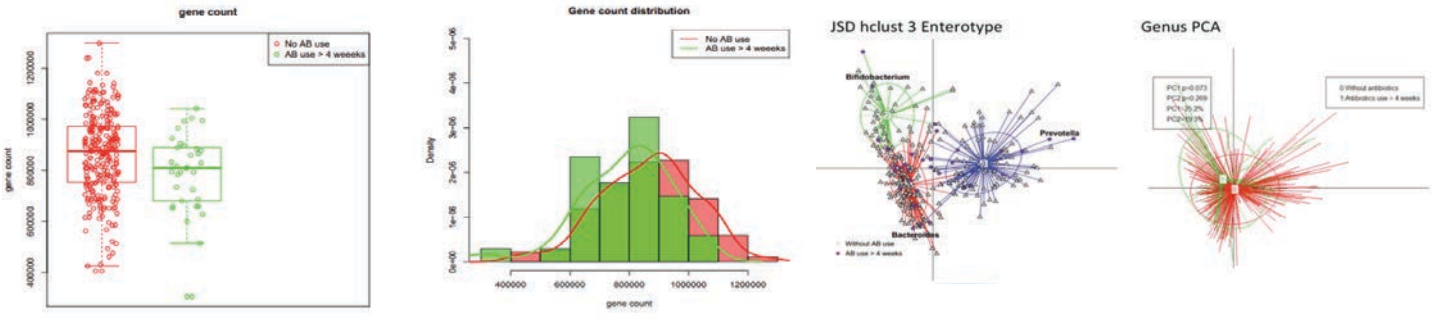
No. of courses over the entire follow-up period	No. of courses by age	No. Courses by type of antibiotics
- None	- 0-6 months	- Broad spectrum β-lactams
- 1	- 6-12 month	- NS β-lactams
- 2-3	- 1-2 years	- Antimetabolites
- ≥4	- ≥ 2 years	- Macrolides
		- Others

One of the initial aims was to assess body weight and BMI z-scores and overweight (as a binary variable) in 979 children with anthropometric data (measured repeatedly across ages 1 – 10 years) in relations to antibiotic usage as a major risk factor in altering the microbiota composition. This exposure about variable was based on antibiotic prescription data collected from general practitioners over the first 10 years of life. This included information on the number of courses of antibiotics across the entire period, and by age grouping. The type of antibiotic, which will have different effects on microbial communities was also analysed. Their hypothesis was that antibiotics and oral hygiene may lead to shift in the state of microbial communities (as proposed by *Costello EK et al. Science 2012*) and this may have consequences for developing metabolism and weight gain. The KOALA findings are consistent with this. Initial analysis suggests that more than two courses of antibiotics in the second year of life was associated with increased weight z-scores. This was not seen over two years of age. And in the first year of life one course of antibiotics was also associated with increased weight z-scores. This suggest that repeated exposure to antibiotics, especially broad-spectrum β-lactams is associated with weight z-scores.

They went on to perform **compositional microbiome analyses using** qPCR to quantify archea and HITChip to quantify bacteria, including 1140 unique microbial phylotypes (16S-based probes). In initial microbiome analysis, high versus low *Prevotella* abundance explains most of the variability in enterotypes. Other individual characteristics did not explain much of the variation in the microbiota composition, although breastfeeding was a factor influencing genus composition.

An additional focus on archaea (in addition to bacteria) was based on the importance of Methanogenic archea removing excess hydrogen and facilitating energy extraction. Accumulation of hydrogen in the gut reduces the efficiency of microbial fermentation as well as the yield of energy. Methanogenic archaea are therefore particularly significant for the human gut, because they are pivotal in the removal of excess hydrogen. Archaea are most prominently represented by the methanogenic *M. smithii* which is the most common methanogenic archaeon in the human gut microbiota. Although in *M. smithii* is paramount in digestive processes, it has a low prevalence in human faeces. *M. smithii* has significant enrichment of genes involved in the utilization of CO_2, H_2, and formate for methanogenesis. *M. smithii* is believed to be a therapeutic target for manipulation and an adaptation to the gut ecosystem. Accordingly, analysis of 472 KOALA children with repeated anthropometric data measured at ages 6-10 years, revealed presence/higher abundance of *M. smithii* in the gut of children (age 6 – 7 years) was associated with: increased weight z-scores and increased risk of being overweight. *Akkermansia, Sutterella wadsworthia*, and *Bryantella formatexigens* were all consistently inversely associated with the three anthropometric outcomes, even after adjusting for the presence of other gut microbial groups & confounders. *Bryantella formatexigens* et rel. has been shown to ferment glucose to acetate in the presence of high formate concentrations and the production of acetate can result in appetite suppression, suggesting a mechanism by which these bacteria might be linked to lower weight.

Consistent with the effects of antibiotics on weight gain patterns (above) they were also fracture on the underlying microbial composition in childhood. This figure shows the impact of antibiotic used for more than four weeks in the preceding year, with general description of microbiome and metagenome stratified for antibiotics use (n=34) or not (n= 269).



In summary, antibiotic use in early life is associated with weight development in childhood. The first 6 months of life appear to be the most susceptible time-window. Archea and several key bacterial genera (e.g. Akkermansia) are associated with childhood weight even in within a relatively lean population. Preliminary metagenome analysis shows a relatively high bifidobacterial abundance in school-aged children, with an associated reduction in gene count and reduction in bifidobacteria related to AB exposure. Gene and genus composition still strongly affected by breastfeeding. The also found some early indications for associations with metabolic outcomes in children.

PROPOSAL: A new in-FLAME Global Environment Working Group

Providing an integrative systems framework for understanding the eco-biological impact of living environments (including biodiversity) on microbial diversity and life-course human health from the perspective of omics and nature relatedness. This proposal for a new working group, led by Karsten Kristiansen and Alan C Logan, emerged during the Early Environment Session, based on the need for abroad and integrated framework that can unify all aspects of the inflame network:

Our mandate:

To develop our profile and new projects around the **impact of dysbiotic drift on global health**, as a common unifying link to many aspects of health and environmental exposures. Given the multisystem impact of the environment through immune health, particularly in early life, the in-FLAME group of experts are well positioned in this area.

Background:

Major themes at the 5th Annual Meeting of the International Inflammation Network included systems biology, molecular biomarkers (including those derived from the microbiome) and the relationship between built/natural environments and NCDs. The **Global Environment Working Group** was established to explore ways in which the use of objective markers might illuminate epidemiological findings (e.g. green space and health outcomes) and psychological constructs (e.g. nature relatedness, positive emotions) associated with health and well-being.

To achieve this aim, and to better understand the mechanisms supporting these relationships, the Working Group discussions included expert opinion on an number of key domains that in-FLAME is well placed to contribute (including the development of integrated focused projects):

1. The use of cohorts (existing and planned) to gather larger data sets; may also involve retroactive research on older cohorts using participant postal codes matched to objective vegetation indices of green space (e.g. Normalized Difference Vegetation Index (NDVI), commonly used global-based vegetation index or Geographic Information Systems (GIS) which provide more information on accessibility).
2. The use of omics – genomics, metagenomics, proteomics, metabolomics
3. The development of animal models that might explain mechanistic pathways of environmental constituents (e.g. the untold influences of cutaneous and/or airborne microbiota on systemic immune function and behavior)
4. The application of systems biology to determine predictive value of potentially salient interacting components
5. The application of short-form mental health (e.g. WHO-5*) and psychological scales (e.g. NR-6**)

*Topp CW, et al. The WHO-5 Well-Being Index: a systematic review of the literature. Psychother Psychosom. 2015;84(3):167-76.
**Nisbet EK, et al. The NR-6: a new brief measure of nature relatedness. Front Psychol. 2013 Nov 1;4:813.

The **Global Environment Working Group** will work toward the primary goals of in-FLAME. That is, to address the risk factors, pathways and strategies to overcome the rising propensity for chronic inflammatory disorders.

Rationale for establishing this Working Group

An increasing body of research has found associations between residential proximity to green (and blue) space and healthy pregnancy outcomes, NCD risk and good mental health [1-6]. These associations are more robust in areas of socioeconomic disadvantage and they are not exclusively explained by use of green space, physical activity or air quality [7-9]. Small-scale field studies indicate that time spent in natural environments (vs. urban built environment) is associated with lower inflammatory cytokines/chemokines [10]. However, there are many questions surrounding the epidemiological associations; at present there is lack of insight into physiological mechanisms that might help explain NCD risk reduction.

Concurrent with the expanding green space research, there is also an emerging area of research linking psychological constructs such as nature relatedness [11] and positive emotions (optimism, joy awe) to health and well-being [12,13]. Although limited, there is evidence that such emotions are associated with a lower inflammatory burden and reduced NCD risk [14-16]. However, once again, mechanisms remain elusive.

A greater understanding and linkage of these psychological attributes to objective markers, including those in the realm of the microbiome, would help to

uncover interactive pathways. As one example, these may include the complex relationships between pet ownership, microbiota and risk of allergic diseases. How does nature relatedness and positive emotion mediate pet ownership decisions and/or microbiota directly by lowered inflammation and stress burden? How might skin microbiota (or inhaled microbiota) differ in those living in areas with high concentrations of green space, and might these differences influence blood/urinary markers of relevance in the new frontier of molecular biomedicine?

As these questions are answered there will be seeding of data required by systems biology.
There seems ample justification for the Working Group; many outstanding gaps in scientific knowledge could be addressed and novel questions will unfold. Given the importance of this topic in the context of early-life origins of health, global health disparities and environmental issues (biodiversity loss) funding opportunities may exist through novel channels – e.g. Wellcome Trust, Bill and Melinda Gates Foundation, Rotary Foundation Networks – potentially through a coordinated collaborative multilateral partnership approach.

Identifying funding agencies aligned without purpose:
As we develop and articulate our profile in this area (through our intended Workshop Position Statement and other channels) we will be well aligned with the agenda of key funding bodies and other partners who are moving more towards Planetary Health. A good example is the Welcome Trust: Our Planet, Our Health

> *"The health of* ***the global population and the planet are inextricably linked but there is a poor ecological fit between what we are asking of the planet and its resilience. If the complex natural systems we rely on for clean air, fresh water, fertile soil, biodiversity and a stable climate are threatened, so too is our health****. The challenge is to secure the health and well-being of present and future generations whilst responsibly stewarding the planet.*
>
> *"As research continues to unravel our understanding of the vital links between health and the environment, we become better equipped to develop robust, coherent and coordinated solutions that jointly reduce threats to human health and to the surrounding environment that sustains it. There are already clear opportunities for change but more research is needed.*
>
> *"We want to support work that embraces and stimulates the formation of creative partnerships because we believe that a diversity of competencies is required to tackle these complex problems. The aim for the initiative is to gain deeper insights into the issues to inform the global response through transdisciplinary research, and develop policies that will help mitigate the risks to human health.*
>
> ***We support transdisciplinary research that connects environment and health."***

The next steps: are to identify groups/ individuals who can contribute to the 5 points made above including:

- Which cohorts can integrate NR questions and additional outcome questions, analysis (see above). Ideally these have biological samples that can be used for biological studies
- Tandem animal models that can address mechanistic pathways (under discussion)
- Development of funding proposal based on the program of research that arising from these further and more detailed discussions and feedback.

Once we have additional feedback we will proceed to the next stage, which is a more detailed document, with contributions from specific groups as required.

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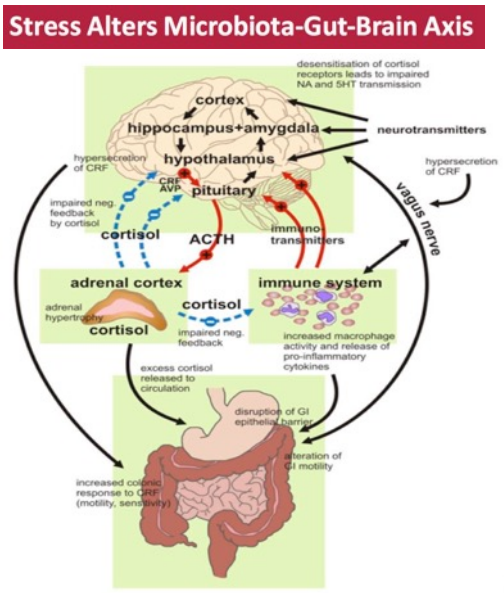
The microbiome, inflammation, behaviour and mental health:

A combination of dietary prebiotics and the probiotic LGG modulate behavioural and cognitive reponses to early life stress

Siobhain M O'Mahony , Karen-Anne McVey Neufeld, Rosaline V Waworuntu, Brian M Berg, Timothy G Dinan, John F Cryan.

Maternal separation (MS) of rat pups is a robust and reliable model of early life adverse events that induces long-term alterations to behavior and brain neurochemistry. These changes are particularly apparent with respect to the microbiota-gut-brain axis. Since dietary factors are known to impact the gut microbiota, this study assessed the impact of consuming prebiotics polydextrose (PDX) and galactooligosaccharide (GOS) with or without the probiotic *Lactobacillus rhamnosus GG* (LGG) on cognition, social- and anxiety-related behaviors in rodents. Rats were separated from their mothers between postnatal days (pd) 2 to 12 as described in O'Mahony et al., 2009. Both MS and NS rats (N=5-9 each) were fed control or prebiotic diet (7 g/kg PDX-GOS) with or without LGG (108 cfu/ml) in drinking water from pd21 throughout behavioral testing to pd100. No differences in body weight or food intake was noted across diets. However, the open field test revealed that MS rats traveled a shorter distance with reduced velocity compared to the NS rats ($p<0.05$). The effects or early life stress were ameliorated by prebiotic feeding ($p<0.01$) and LGG ($p<0.01$), but intriguingly not when combined. MS rats displayed deficits in spatial memory in the Morris water maze ($p<0.05$) while rats fed prebiotic in addition to LGG showed a reversal of this impairment ($p=0.05$). All diets reduced Glucocorticoid Receptor (GR) mRNA levels in non-stressed rats, however LGG was only diet to increase levels in stressed rats ($p<0.05$) (A). Combination of prebiotic with LGG ameliorated the MS-induced up-regulation of hippocampal GABA A2 receptor mRNA ($p<0.05$). In conclusion, these results demonstrate that both prebiotics and LGG ameliorate early life stress-induced changes in adult behavior and when combined can improve memory performance. These results aid to further our understanding of the effects of dietary manipulation on the microbiota-gut-brain axis in an animal model of early life stress.

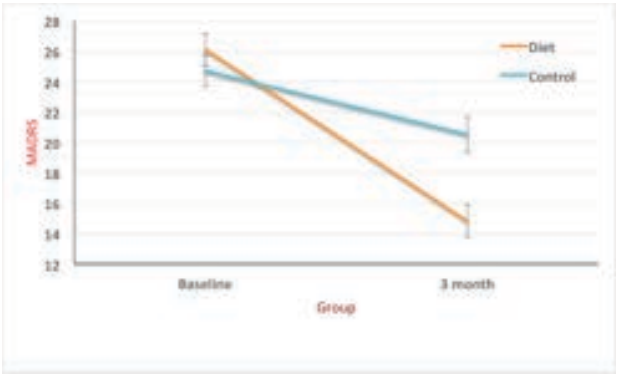


A randomised, controlled trial of a dietary intervention for adults with major depression (the "SMILES" trial)

Felice N Jacka, Adrienne O'Neil, Catherine Itsiopoulos, David Castle, Rachelle Opie, Sarah Dash, Sue Cotton, Cathrine Mihalopoulos, Laima Brazionis, Allison Hodge, Michael Berk

There is now extensive observational evidence across countries, cultures and age groups to suggest that diet and nutrition play a role in the genesis of depression. However, there are currently few data regarding the possible therapeutic impact of dietary changes on existing mental illness. Using a randomised controlled trial design we aimed, for the first time, to investigate the efficacy of a dietary improvement program for the treatment of Major Depressive Episodes (MDE). Participants suffering from current MDE were randomised into a dietary intervention group or a social support group. Depression status was assessed using the Montgomery–Åsberg Depression Rating Scale (MADRS) and Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (Non Patient Edition) (SCID-I/NP). The intervention consisted of 7 individual nutrition consulting sessions (of approximately 60 minutes), delivered by an Accredited Practising Dietitian (APD). Sessions commenced within one week of baseline assessment.

The intervention focused on advocating a healthy diet based on the Australian Dietary Guidelines and the



Dietary Guidelines for Adults in Greece. The control condition comprised a befriending protocol using the same visit schedule and length as the diet intervention. The study was conducted at two locations in Victoria, Australia (a metropolitan and regional centre). Data collection occurred at baseline (pre-intervention), and at 3 - and 6 - month post-intervention. The primary endpoint was MADRS scores at 3 months. 30% in dietary intervention achieved clinical remission compared with 9% in control group (Figure 1 - effect size 0.76, $p<0.001$). This is the first randomised controlled trial to seek to answer the question 'If I improve my diet, will my mental health improve?" The results of this study suggest that dietary improvement with a clinical dietitian may provide an efficacious alternative or adjunct treatment strategy for the management of this highly prevalent mental disorder, the benefits of which could extend to the management of common co-morbidities including cardiovascular disease (CVD), obesity, and type 2 diabetes.

Maternal depression, birth weight and fecal metabolites at 3 months

Manjeet Kumari, Petya Koleva, Rupasri Mandal, Tedd Konya, Sarah Bridgeman, David Wishart, David Guttman, Malcolm Sears, Allan Becker, Piush Mandhane, Padmaji Subbarao, Stuart Turvey, James Scott, Anita Kozyrskyj, and the CHILD study investigators.

Both birth weight and newborn gut microbiota can be shaped in utero by maternal exposures, and have been associated with child overweight and atopic disease. Maternal prenatal depression has been linked with these noncommunicable diseases as well. Gut microbiota produce metabolites which are obesogenic or leptogenic. We undertook a descriptive study of maternal prenatal depression, birth weight and the infant gut metabolome. The study comprised a subset of 190 mothers and their full term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort. Birth weight (range 1590- 4837 g) and maternal prepregnancy overweight (41%) what are extracted from hospital records. Prenatal depression status (28%) and other maternal characteristics were obtained from questionnaires. Infant fecal metabolite composition was ascertained at three months of age using nuclear magnetic resonance (NMR). Our results highlight the influence of prenatal depression on birth weight and guts microbial metabolism months of age. Similar differences in fetal lactate and fatty acid metabolite levels have been observed in overweight children.

KEY FINDINGS

Infants with the lowest tertile bodyweight (median 2990 g) where are more likely to be born to mothers with depression; their mothers were less likely to be overweight and of Caucasian status. At 3 months of age faecal samples of these infants had the lowest concentrations of lactate and the highest concentrations of acetate, butyrate and branch-chain fatty acids. No mode of birth differences were observed across birth weight but infants with the low was birth weight were less likely to be exclusively breastfed.

Diet, nutrition, inflammation and metabolism:

Association of leptin and adiponectin in human milk with maternal body composition

Sambavi Kugananthan[1], Zoya Gridneva[1], Ching Tat Lai[1], Anna R. Hepworth[1], Peter J. Mark[2], Foteini Kakulas[1], **Donna T. Geddes[1]**

Obesity is an increasing prevalent chronic disease in adults and children, and has been linked to unfavorable developmental programming of appetite signaling. Appetite hormones in human milk (HM) such as leptin and adiponectin, which are partially derived from maternal adipose tissue, are proposed to mediate short and long-term infant satiety. The aim of this study was to determine if maternal adiposity was associated with HM leptin and adiponectin. Pre- and post- feed HM samples were collected from 61 Western Australian mothers at 2, 5, 9 and 12 months postpartum. Maternal body composition was measured by multiple frequency bio-electrical impedance at each sample collection. Leptin and adiponectin levels were measured in whole milk using ELISA.. Maternal adiposity likely plays a role in regulating the levels of HM leptin. Although leptin levels are higher in the milk of obese women, their infant may not receive increased total leptin due to the low milk production often suffered by these women. This could have implications for the development of the hypothalamic appetite circuitry, lowering satiety responses in these infants. Further, the fetus of an obese woman may develop leptin resistance *in utero*, due to constant exposure to high maternal serum leptin levels, which may continue during breastfeeding. Appetite hormones in HM could exert synergistic and/or antagonistic effects with other milk bioactive factors that have yet been investigated.

KEY FINDINGS

Leptin levels in whole HM (0.52±0.001 ng/mL) were significantly higher (P<0.0001) than in skim HM (0.27±0.01 ng/mL) and were not associated with milk fat. An increase in leptin levels (0.004±0.002 ng/mL) was observed for every 1% increase of maternal fat (P=0.01). There was no association between adiponectin and maternal fat mass. Overall, leptin and adiponectin levels in whole HM did not differ between pre- and post-feed (P>0.05) and remained constant throughout the stages of lactation (P>0.05)

Dietary fibers and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways

Jian Tan[1], Charles Mackay[1,2] **Laurence Macia[1,3,4]**

Incidence of food allergies in Western countries has increased dramatically in recent decades, for unknown reasons. Significant changes in western lifestyle, particularly consumption of processed food low in dietary fibers might be involved in this phenomenon. Tolerance to food antigens relies on mucosal CD103$^+$ dendritic cells (DCs), which promote differentiation of Tregs. We show that the bacterial metabolites short-chain fatty acids (SCFAs) derived from microbial fermentation of dietary fiber, are important for enhancing the tolerogenic function of CD103$^+$DCs. SCFAs as well as a gut microbiota shaped by dietary fiber enhanced CD103$^+$ DC capacity to promote differentiation of Treg cells. High-fiber diet altered gut microbial ecology, which enhanced oral tolerance and protected against food allergy. Dietary fiber also enhanced TFH cells leading to greater IgA production. Mice deficient in GPR43 and GPR109a, receptors for SCFAs, showed exacerbated food allergy responses, higher IgE production, and fewer CD103$^+$DCs. Thus, dietary elements including fiber and Vitamin A regulate tolerogenic pathways in the gastrointestinal tract upstream of Treg cells, necessary for immune non-responsiveness to food antigens.

Towards the development of a food frequency questionnaire to assess dietary microbial exposure in Dutch adults

Berber Vlieg-Boerstra, Jeanne de Vries, Esther de Jong, Aline Sprikkelman, Hanneke Oude Elberink, Marlou de Kroon, Marcel Zwietering.

Studies have shown that a western life style leads to changes in intestinal microbiota which are associated with lack of immunologic tolerance development and the subsequent development Non-Communicable Diseases (NCDs), including allergies. Diet is an important determinant of the intestinal microbiota composition both by providing nutrients and fiber and by providing foodborne microbes. During recent decades the consumption pattern in the western world has shifted from home-made meals made from fresh ingredients towards ready-to-use and convenience meals and highly processed foods. This may have resulted, as we hypothesize, in a shift in the total consumption of foodborne microbes. Thirty atopic Dutch male/female adults will be randomized to a microbe-rich diet (~10^{11} microbes) or a microbe-poor diet (~10^{5} microbes), during 6 weeks. Ten Dutch atopic breastfeeding mothers will be randomized to a microbe-rich and, for ethical reasons, microbe-moderate (~10^{5}-10^{9} microbes) diet. Additionally, the nutritional composition of the diet will be assessed by 3-day food diaries. Intestinal microbiota composition by 16S sequencing, determination of the general immune status in blood and breast milk, sensitization, quantification and duration of residence of foodborne microbes in saliva, stools and breast milk, quantification of foodborne microbes in the diet.

KEY AIMS

This proof-of-concept study is designed to assess the influence of a diet a. rich, b. poor or c. moderate in foodborne microbes on the microbiota composition of the gut and breast milk, and on sensitization and general immune status in Dutch atopic adults and atopic breastfeeding mothers. The results will sustain our novel and beyond the state-of-the art hypothesis that the presence and diversity of foodborne microbes in fresh, freshly stored and fermented foods, which are absent or reduced in highly processed and heat-treated foods, have immunomodulatory effects and may be preventive for the development of allergies and other NCDs

Increased maternal pre-pregnancy body mass index is associated with increased birth weight, adiposity and inflammation in the newborn

Kate McCloskey, David Burgner, Fiona Collier, Katy Allen, Mimi Tang, John B Carlin, Richard Saffery, Michael R Skilton, Michael Cheung, Sarath Ranganathan,Terence Dwyer, Anne-Louise Ponsonby, Peter Vuillermin

Excess adiposity and adiposity-related inflammation are known risk factors for cardiovascular disease in adults, but little is known regarding adiposity-related inflammation at birth. Although increased maternal body mass index (BMI) is directly associated with both inflammation in pregnancy and birth weight, the relationships between maternal BMI and offspring adiposity and inflammation are unknown. The aim of this study was therefore to investigate the association between maternal pre-pregnancy BMI and newborn adiposity and inflammation. 161 paired maternal (28-weeks gestation) and newborn (umbilical cord) blood samples were selected from a population-derived birth cohort (Barwon Infant Study, n=1074). Data on maternal co-morbidities, newborn birth weight and adiposity (standardised skinfold thickness) were compiled. Maternal high-sensitivity C-reactive protein (hsCRP) was measured during third trimester and in cord blood. Higher maternal pre-pregnancy BMI is associated with increased newborn adiposity and inflammation. These associations may be partially mediated by maternal inflammation during pregnancy.

KEY FINDINGS

Maternal pre-pregnancy BMI was positively associated with increased birth weight (adjusted mean difference =17.8g per kg/m^2, 95%CI 6.6 to 28.9; p=0.002), newborn mean skinfold thickness, (adjusted mean difference =0.1 mm per kg/m^2, 95%CI 0.0 to 0.1; p<0.001), and cord blood inflammation (adjusted mean difference of 4% increase in cord hsCRP per 1kg/m^2 increase for pre-pregnancy BMI, 95%CI 1 to 8%, p=0.02). Inclusion of maternal hsCRP during pregnancy as a covariate attenuated the associations between pre-pregnancy BMI and cord blood hsCRP.

Dietary GOS prevent eosinophilic inflammation in HDM-model: role of Treg.

Kim Verheijden; Saskia Braber; Thea Leusink-Muis; Suzan Thijssen; Louis Boon; Aletta Kraneveld; Johan Garssen; Gert Folkerts; Linette Willemsen

Dietary non-digestible galacto-oligosaccharides (GOS) have been shown to suppress allergic symptoms. Previously, $CD25^+$ regulatory T-cells (Treg) were found to contribute to allergy protection induced by non-digestible oligosaccharides. Now, we investigated the effect of anti-CD25 Treg depletion in a murine HDM-induced asthma model and studied the contribution of Treg in the protective effect of dietary intervention with GOS. BALB/c mice were intranasally sensitized and challenged with HDM or PBS while being fed a control or a 1 w/w% GOS diet. Treg were depleted by two intraperitoneal injections with anti-mouse CD25 antibody. T-helper (Th) cell subtypes in lung and spleen of control diet fed anti-CD25-treated mice were analyzed by flow cytometry and cytokines were measured in re-stimulated lung cell supernatants. Leukocyte subtypes were analyzed in the bronchoalveolar lavage fluid (BALF) and interleukin 33 (IL-33) and chemokines measured in lung homogenate supernatants. Dietary intervention with GOS abrogated the increase in IL-33 which was abolished by the anti-CD25 treatment, CCL5 showed the same tendency. Treg suppress HDM-induced asthma in mice. Dietary intervention with GOS has a beneficial effect on the prevention of HDM-induced allergic asthma by supporting pulmonary Treg function.

KEY FINDINGS

Anti-CD25 depleted $CD25^+Foxp3^+$Treg in lung and spleen of control and HDM-allergic mice, while the frequency of activated T helper cells and Th2 cells increased. This was associated with increased IL-10, IL-4 and IL-13 concentrations in lung cell supernatants. BALF leukocyte numbers and percentages of eosinophils and lymphocytes were increased in HDM-allergic mice but remained unaffected by the anti-CD25 treatment. The GOS diet decreased airway eosinophilia, this protective effect was lost in anti-CD25 treated mice. A similar pattern was observed for lymphocytes and neutrophils. In lung homogenate supernatants of HDM-allergic mice, IL-33 and CCL5 concentrations were increased compared to control).

Reduction in allergic features in offspring of mice supplemented with specific non-digestible oligosaccharides during lactation

Astrid Hogenkamp[1], Suzan Thijssen[1], Leon Knippels[1,2], Johan Garssen

Maternal supplementation with non-digestible carbohydrates during pregnancy has been shown to reduce the development of several allergic asthma features in adult offspring. In the current study, it was investigated whether maternal supplementation during lactation only would have similar effects. After two weeks of acclimatization, the breeding protocol was started. Directly after birth of the offspring, mice in the intervention group were transferred to AIN93 control diet supplemented with short-chain galacto- and long-chain fructo-oligosaccharides (scGOS/lcFOS; ratio 9:1). Male offspring were sensitized to OVA at 6 weeks, and acute allergic skin responses (ASR) upon intradermal ovalbumin challenge were measured at the age of 8 weeks. Airway hyperreactivity (AHR) to metacholine was measured after 3 consecutive airway challenges with OVA. Specific plasma immunoglobulins were measured in blood; T-cell populations were analyzed in spleen and thoracic lymph nodes and cell populations in bronchoalveolar lavage fluid (BAL) were assessed. Maternal supplementation with scGOS/lcFOS during lactation down-regulated allergic inflammation in the lungs. Immunoglobulin levels, relevant for allergic disease, were down-regulated as well. In contrast, allergic skin reactions and lung functions were not affected. Our data suggest that early life dietary intervention with non-digestible carbohydrates may be beneficial for the allergic outcome later in life, which is also highly relevant for the development of atopic disease in humans.

KEY FINDINGS

ASR and AHR did not differ significantly between the control and the intervention group, but allergic inflammation was significantly down-regulated by the dietary intervention during lactation. Total cells numbers, and percentages of eosinophils and lymphocytes in BAL as markers for allergic inflammation were significantly decreased in the intervention group. OVA-specific and total IgG1 levels were significantly lower in the intervention group. Although OVA-specific IgE levels did not differ between the intervention and control group, levels of total IgE were significantly lower in the intervention group.

Dietary targeting of maternal gut health for better child outcomes: the healthy parents, healthy kids RCT protocol

Samantha Dawson, Jeffrey Craig, Mimi Tang, Gerard Clarke, Felice Jacka

Maternal diet quality is associated with mental health outcomes in children. Pre-clinical studies indicate that offspring brain development and function may be disturbed by perturbations to inflammatory status or gut microbiota. Epigenetic regulation and HPA-axis function may also be important mediators of brain development and function. The western diet is associated with microbial dysbiosis and inflammation, whereas quality nutrient-rich, high-fibre diets are associated with greater microbial diversity and reduced inflammation. We hypothesise that dietary improvement during pregnancy will beneficially influence these underlying factors in mothers and their children. Our aims is to assess the feasibility and efficacy of a prenatal educational dietary intervention in influencing gut microbial diversity and metabolites, markers of inflammation, epigenetic markers, and cortisol in mothers and their infants post-birth. Ninety pregnant women will be recruited and randomised to either continue receiving treatment as usual from their healthcare provider, or to receive an intervention designed to promote gut health in mothers during the third trimester of pregnancy and the early prenatal period. The intervention involves an educational dietary workshop promoting the Australian dietary guidelines and increased intakes of prebiotic and probiotic foods, plus two phone calls to support adherence. Exclusion criteria preclude: ages under 18; mental illnesses; obesity; diabetes mellitus; bowel conditions; medically-advised exclusion diets; illicit drug use; recent antibiotic use; recent pre/probiotic supplementation; and those lacking dietary autonomy. The efficacy of the intervention in improving outcomes will be evaluated by analysing between-group differences in: dietary intake, microbial diversity and richness; short chain fatty acids; epigenetic profile; and markers of inflammation and stress in mothers and neonates. These data will be used to provide new insights regarding the potential of targeting the maternal diet to improve maternal and infant parameters that are relevant to mental health outcomes in children.

Developing effective strategies to improve pregnancy and neonatal outcomes in youth with type 2 diabetes

Aveni Haynes, Mark Shah, Jacqueline Curran, Elizabeth A Davis.

As the incidence of Type 2 diabetes continues to increase worldwide in youth prior to their childbearing years, so does the prevalence of pregnancies complicated by this chronic, non-communicable disease. As well as a higher risk of preterm birth and congenital anomalies, infants born to mothers with Type 2 diabetes have an increased risk of metabolic and immune dysfunction health outcomes, including obesity and Type 2 diabetes, in later life.

Our study will be undertaken at the Child and Adolescent Type 2 diabetes clinic at Princess Margaret Hospital, the only tertiary paediatric endocrinology centre in Western Australia, which provides multidisciplinary diabetes-related health services to 194 youth diagnosed with Type 2 diabetes. Currently, there is no regular counselling provided on the need for, or importance of, contraception and adequate pre-conception health care, including optimal glycaemic control and body weight. In collaboration with local obstetric hospital services and diabetes nurse educators, age-appropriate tools will be developed to facilitate annual patient education and counselling regarding these issues. In the future, this study will provide important data for a follow-up study assessing the rates of teen-pregnancies, use of contraception and outcomes of future pregnancies in youth with Type 2 diabetes in a contemporary, population-based cohort. This will be enabled by using data-linkage with State-wide data resources available in Western Australia, such as the Midwives' Notification System and the Western Australian Birth Defects Registry.

KEY AIMS

This study aims to develop pre-conception and contraception counselling strategies in a clinical setting to educate and empower youth with Type 2 diabetes regarding pregnancy avoidance and/or optimal pre-conception and antenatal care and improve pregnancy and neonatal outcomes in this high risk population, thereby reducing the rising burden of this disease. A secondary aim is to estimate the proportion of births in Western Australia whose mothers have a diagnosis of Type 2 diabetes at the time of birth.

Allergic inflammation and immune development:

Allergic and non-allergic childhood asthma is characterized by novel gene expression profiles and signaling pathways

Andreas Böck, Diana Rädler, Olivia Prazeres da Costa, Katja Landgraf-Rauf , Elisabeth Klucker , Erika von Mutius, Thorsten Buch, Ulrich Mansmann, **Bianca Schaub**

Asthma is a complex immune-mediated disease truly relevant as the most prevalent chronic disease in childhood worldwide. Different asthma phenotypes have been reported. However their distinct immune pathogenesis is still not well defined.To identify specific biomarkers and cellular pathways for the differentiation of allergic (AA) and non-allergic asthmatic (NA) children in comparison to healthy controls (HC). In the CLARA study population (n=315) peripheral blood mononuclear cells (PBMCs: n(AA/NA/HC) = (14/8/14)) and $CD4^+$ cells of a subset of children, comparable to the whole population, were stimulated with anti-CD3/CD28, LpA or kept unstimulated. Gene-expression was investigated by transcriptomics (n=105 arrays) and quantitative real-time RT-PCR. Microarray data were analyzed with explorative screening methods using empirical Bayes linear modelling and confirmatory procedures applying global tests of differentially expressed cell-type-associated and pathway genes. Childhood allergic and non-allergic asthma was differentiated by novel specific gene expression profiles, suggesting unique concepts of immune mechanisms. These open novel perspectives for prognosis, phenotype-specific therapies and treatment response.

KEY FINDINGS

AA were characterized by differential transcriptional regulation of mTOR signaling with increased expression of Treg-associated genes and decreased expression of *IRF8*, involved in intracellular defence against pathogens. NA were characterized by differential transcriptional regulation of TLR signaling and increased expression of genes associated with, airway remodeling (*ETS2*, *HNMT*) or *S100A8 and S100A9 expression* compared to AA. Expression of Th2 genes was increased in AA and NA compared to HC. Microarray analysis of PBMCs identified more differentially regulated pathways than analysis of isolated $CD4^+$ cells.

IgA and local mucosal responses to gut microbiota and the risk of infant allergy

Majda Dzidic, Thomas Abrahamsson, M Carmen Collado, Bengt Björkstén, Alex Mira, Maria C Jenmalm

The increasing allergy prevalence in affluent countries may be caused by reduced exposure and diversity of microbial stimulation, resulting in abnormal postnatal mucosal immune maturation. While a reduced gut microbiota diversity and low mucosal total IgA levels in infancy have been associated with allergy development, IgA responses to the gut microbiota have not been studied. The proportion of the gut microbiota, bound to IgA or not, was analyzed by flow cytometry-based sorting of faecal samples, collected at 1 and 12 months in 20 children developing allergy and 28 without allergy to 7 years of age. The microbial composition and diversity of bacteria, bound or not bound to IgA, were analyzed with barcoded 16S rDNA 454-pyrosequencing. Total secretory IgA and bacterial load of the stool samples of healthy and allergic children was also determined. Allergy and asthma development associates with an altered IgA responsiveness to the gut microbiota during infancy, possibly indicating an impaired mucosal barrier function.

KEY FINDINGS

IgA-coating patterns decreased from 1 to 12 months in both allergic children and children staying healthy up to 7 years of age, reflecting the contribution of maternally derived IgA antibodies in breast milk during. Children developing allergic manifestations, particularly asthma, during childhood had a lower proportion of IgA bound to faecal bacteria at 12 months of age compared to healthy children. This cannot be attributed to differences in IgA levels nor to differences in bacterial load, which was shown to be higher in healthy children. However, the bacterial targets of early IgA responses (including the coating of genus *Bacteroides*) as well as the IgA recognition patterns, detected by Principal Component Analysis, seem to differ between healthy children and children developing allergic manifestations.

Differential loading of IgE on circulating CD19$^-$cKit$^+$CD38$^+$ cells between atopic and non-atopic individuals

Catherine Li Lai, Brigitte Nanan, Ming Jing Hu, Peter Hsu, Dianne Campbell, Ralph Nanan

IgE-mediated food allergic reactions are commonly elicited by food allergens binding to surface IgE attached to high affinity FcεRI receptors on mast cells. In affected tissues mast cells release pre-formed granules containing mediators such as histamine, triggering local vasodilation and increased blood flow and determine some clinical features of allergic reactions. In this study we compared a distinct subset of IgE$^+$CD19$^-$c-Kit$^+$CD38$^+$ cells, suggestive of circulating mast cells, between non-atopic controls and egg allergic patients. Peripheral blood mononuclear cells were analysed in children with confirmed egg allergy (n=8) and in non-atopic controls (n=10). Multiparametric flow cytometry was used for phenotyping of PBMC with a panel of fluorescence conjugated monoclonal antibodies. Non-parametric Mann-Whittney tests were used to detect statistical differences between groups. These findings suggest that IgE$^+$CD19$^-$cKit$^+$CD38$^+$ cells in peripheral blood differentially express FcεRI receptor in atopic and non-atopic individuals. The phenotype of these cells suggests that they are small circulating mast cell precursors binding IgE via the high affinity FcεRI receptor. A correlation of these cells with clinical severity might be warranted in future studies to analyse their predictive value in food allergies.

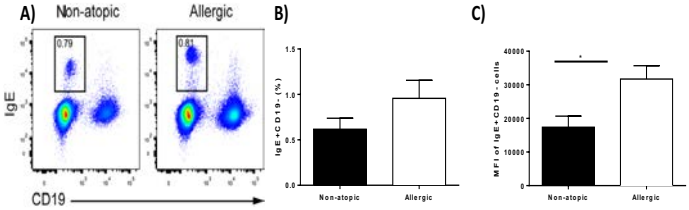


Figure 1. IgE$^+$CD19$^-$ cells in non-atopic and egg allergic children. **(A)** Representative dot plots of IgE$^+$CD19$^-$ cells. **(B)** Comparison of IgE$^+$CD19$^-$ cells between groups for frequency and **(C)** mean fluorescence intensity (MFI). * $p < 0.05$.

KEY FINDINGS

A clearly distinct population of IgE$^+$CD19$^-$ cells was detected, which was strictly confined within the lymphocyte gate. These cells also expressed c-Kit and CD38 and were negative for CD14, CD20, CD21, CD23 and CD56. In contrast to IgE$^+$CD19$^+$ B cells, which were either positive for λ or κ Ig light chain, IgE$^+$CD19$^-$ cells were all double positive for λ and κ. The frequency of this subset varied between individuals but was not statistically different between the groups (Figure 1A and B). However, the mean fluorescence intensity of cell surface IgE was significantly higher in the allergic group compared to non-atopic controls (Figure 1C).

in-FLAME

Metabolomics profile of amniotic fluid and early recurrent wheezing

Silvia Carraro, Giuseppe Giordano, Paola Pirillo, Mauro Naturale, Matteo Stocchero, Michiel Houben, Eugenio Baraldi and Louis Bont

Whether early recurrent wheezing depends on the type of triggering viruses or whether an underlying predisposing condition exists, is still a matter of debate. Some data suggest a pre-existing abnormal lung condition, but the reasons of such predisposition are not known. Metabolomics is the analysis of low molecular weight metabolites in biological samples through spectroscopic techniques. Guided by no "a priori" hypothesis, metabolomics leads to the identification of metabolic patterns characteristic of a specific pathological condition.Our aim was to apply the metabolomic approach to evaluate whether specific biochemical-metabolic characteristics of the amniotic fluid at birth are associated with recurrent wheezing during the first year of life. We analyzed 147 amniotic fluid samples collected at birth in newborns delivered at term. The metabolomic analysis was performed through Mass Spectrometry (QTof Synap G2 HDMS, Waters) combined with Ultra-Performance Liquid Chromatography (UPLC, Acquity, Waters). The obtained spectroscopic data were extracted with Markerlynx (Waters) and subsequently examined with multivariate statistical analysis tools. The metabolomic analysis of amniotic fluid at birth enabled the discrimination of infants who developed recurrent wheezing during the first year of life. Our results suggest an early imbalance between oxidants and anti-oxidants, already present during intra-uterine life, as a possible pathogenetic mechanism involved in recurrent wheezing.

KEY FINDINGS

Constrained Projection to Latent Structures-Discriminant Analysis (CPLS2-DA) was used to exclude the effects on data modeling of several crucial clinical variables. The CPLS2-DA model, build upon stability-based variable selection, was able to discriminate between infants with (n=57) and without (n=90) recurrent wheezing. The search of the available online databases for the main metabolites involved in the discrimination, suggests a preponderance of products related to oxidative stress (e.g. indoxyl sulfate) in subjects who developed recurrent wheezing and of anti-oxidant metabolites (e.g. polyphenol derivatives and ascorbic acid) in subjects who did not.

Preeclampsia is associated with reduced regulatory T-cell proportions in infants during the first year of life

Fiona Collier, Anne-Louise Ponsonby, Mimi LK Tang, Ralph Nanan, Peter Vuillermin, The BIS Investigator Group.

Preeclampsia (PE) is a common pregnancy disorder characterized by maternal hypertension, proteinuria and systemic inflammation. Recent studies indicate maternal immune dysregulation in PE also affects fetal immune tolerance with a drop in prenatal fetal thymic size and reduced number of cord blood regulatory T-cells (Treg), however the long-term effects on infant immune cell populations are not known. The aim of the study was to investigate Treg populations over the first year of life of infants born to mothers with PE.The Barwon infant Study (BIS) is a prospective birth cohort with antenatal recruitment (n=1074). The incidence of self-reported PE was 2.6% (27/1047). Two suppressive Treg populations (naïve, $CD4^{+}FoxP3^{+}CD45RA^{+}$ (nTreg) and activated $CD4^{+}FoxP3^{high}CD45RA^{neg}$ (aTreg)) were measured by flow cytometry in a randomly selected group of freshly collected blood samples at birth (n=447), six (n=592) and twelve months (n=653). Regression analysis was used to test the difference in Treg populations of infants from PE mothers and non-PE mothers at each time point. Covariates including gestational age, mode of delivery and sex were considered in the model. These data indicate that PE is associated with a reduced proportion of offspring Treg over the first six months of postnatal life. Studies are required to determine the basis of this association.

KEY FINDINGS

In unadjusted analyses PE was associated with a 16% and 34% reduction in neonatal cord blood nTreg (p=0.038) and aTreg (p=0.038) proportions respectively. At six months the rTreg proportion was 13% lower than non-PE (p=0.077). By twelve months both the rTreg and aTreg in the PE infants were more closely aligned with non-PE levels (Table 1). Longitudinal analysis by generalized estimating equation (accounting for repeated measures) indicated that nTreg were lower in PE infants over the first year of life (-0.47(-0.85, -0.09)%, p=0.016). Inclusion of the covariates made <10% difference to results.

The Influence of Vitamin D and UV Exposure on the Developing Immune Phenotype in Infancy

Kristina Rüter, Anderson Jones, Aris Siafarikas, Ee M Lim, Susan L. Prescott, Debra J. Palmer

Low levels of vitamin D have been linked to allergies in children, the potential result of lifestyle and reduced sunlight exposure. Vitamin D supplementation is already in use in many countries. This has not occurred in Australia, largely due to the belief that children are exposed to adequate sunlight. However, there is growing evidence of suboptimal vitamin D levels and a pressing need to understand if this is a contributing factor to the dramatic increase in infant allergy. In this double-blind, placebo-controlled randomised trial, high risk infants are orally supplemented with either 400 IU vitamin D/day or placebo from birth to 6 months of age. Uniquely a UV-dosimeter is also worn. Blood samples are collected at birth, 3, 6 and 12 months of age to determine relationships between oral vitamin D supplementation and UV-light exposure with blood 25(OH)D concentration, immune cell function responses to allergens and on the development of allergic conditions in early childhood. Early preliminary results from this study demonstrate novel findings that vitamin D levels and UV exposure in infancy have important consequences for the developing immune phenotype in a population at hereditary risk of allergic disease. The findings offer insight into possible immunological mechanisms behind the reported inverse associations between vitamin D status and allergic disease.

KEY FINDINGS

To date initial findings (n = 42) have found that vitamin D and UV exposure, both play a role in maintaining a suppressive Treg pool characterized by a greater expression of HLA-DR and CD45RA, and fewer $CD69^{+}$ cells. Additionally a lower percentage of circulating mature dendritic cells (DC) was found. Beyond this we have found correlations between average daily UV exposure in the first 3 months and DC with reduced propensity for T cell activation at 6 months.

Vitamin-D deficiency augments cytokine expression in murine Th2-cells

Ayşe Kılıç, Matthias Schiller, S Uchida, Mizue Teranishi, P Gellert, Marie Demay, Thomas Braun, Harald Renz

Vitamin-D (Vit-D) possesses immunmodulatory functions and Vit-D deficiency has been associated with severity of chronic inflammatory diseases. In asthmatic patients, this condition is linked to exacerbations and high IgE levels. Studies, assessing the effect of Vit-D supplementation on immune regulation, provide contradictory results and raise the need for deeper analysis of the underlying molecular mechanisms. To investigate the importance of Vit-D and its receptor (VDR) in Th2-driven diseases, we used Balb/c mouse models of Ovalbumin induced allergic airway inflammation (acute and chronic), combined these with FACS-sorting of distinct live T-helper (Th) subsets (Th1, Th2 and Treg) from inflamed lungs and microarray analyses. Functional studies were performed with WT (Vit-D sufficient diet), WT/Vit-D deprived (Vit-D deficient diet from 3rd trimester of pregnancy) and VDR-knockout (KO) mice on C57BL/6J background in the *in vitro* system of differentiating Th-cells.. In conclusion these data indicate an important role of Vit D – Vit D receptor signaling in the regulation of Th2 immunity.

KEY FINDINGS

Microarray results revealed 5-fold higher expression of VDR in Th2 cells compared to naïve T cells, Th1 and Treg cells, implicating a specific function of Vit-D in Th2 cells. In vitro experiments revealed that Th-cells, deficient in Vit-D signaling, displayed lower IFN-γ (Th1) and IL-4 (Th2) levels. In contrast, loss of Vit-D signals in Th2 cells augmented IL-5, IL-6 and IL-13 levels compared to wild type cells. Th2 cells in murine allergic airway inflammation can respond to Vit-D signals. Vit-D is able to mediate cell and cytokine specific effects and is needed for dampening the expression of disease promoting cytokines.

Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce non-classic 'Th2-type' immunity associated with development of food allergy

Yuxia Zhang, Fiona Collier, Gaetano Naselli, Richard Saffery, Mimi L Tang, Katrina J Allan[2], Anne-Louise Ponsonby, Leonard C Harrison and **Peter Vuillermin** on behalf of the BIS Investigator Group

Infants who develop food allergy have increased innate immune activity and decreased FOXP3$^+$ natural regulatory T cell (nTreg) number and function in cord blood. We sought to understand how these findings are related to CD4$^+$ T helper 2 (Th2) cell immunity that characterizes allergy. We assembled a general population-derived birth cohort (n=1074) and characterised IgE-mediated food allergy at 1 year of age. Immune cell composition in cord blood was determined by flow cytometry; innate immune function was measured in CD14$^+$ monocytes as lipopolysaccharide (LPS)-stimulated cytokine secretion; differentiation and function of naïve CD4$^+$ and naïve nTreg were examined by T cell receptor and co-receptor CD28 activation under defined cytokine conditions. A hyper-responsive innate immune state at birth promotes non-classic Th2 differentiation by repressing IL-2, providing a plausible mechanism underlying susceptibility to food allergy.

KEY FINDINGS

Infants destined to develop food allergy demonstrated increased cord blood inflammatory responses to labour. Concordantly LPS-stimulated CD14$^+$ monocytes of infants who developed food allergy secreted higher amount of inflammatory cytokines IL-1, IL-6 and TNF-a. These inflammatory cytokines suppressed IL-2 expression by CD4$^+$ T cells, and in the absence of IL-2, decreased the number of activated nTreg and diverted both nTreg and CD4$^+$ T cells towards a non-classic Th2 type immune phenotype.

Whole genome methylation patterns in circulating CD4+ cells of infants participating in a probiotic intervention study

Johanna Huoman, Anna Forsberg, Ratnesh Bhai Mehta, Bengt Björkstén, Thomas Abrahamsson, Maria C. Jenmalm

Differential DNA methylation patterns may mediate outcomes of perinatal probiotic intervention in children with high risk of developing allergic disease. In our placebo controlled randomized multicenter allergy prevention trial, mothers were supplemented with the probiotic strain *Lactobacillus reuteri* ATCC 55730 ($1x10^8$ CFU daily) from gestational week 36 until delivery and the infants throughout the first year of life. Probiotic intervention reduced IgE-associated eczema at two years, but did not influence respiratory allergies at seven years of age. To investigate the influence of probiotic intervention on whole genome level DNA methylation, genomic DNA was extracted from circulating CD4+ T cells at three time points (n: CB = 29, 12 months = 10, 24 months = 19). DNA methylation patterns acquired utilizing Infinium Human Methylation 450 bead array technology were subsequently analysed in the R programming environment (RnBeads package) and differential DNA methylation patterns were further studied using bioinformatic approaches (DAVID). Perinatal probiotic supplementation seems to modulate epigenetic patterns related to immune function in children with a high propensity of developing allergic disease.

KEY FINDINGS

Preliminary results showed that probiotic intervention resulted in hypomethylation, and hence increased transcriptional activity, of numerous immune function associated biological process clusters at birth. In contrast, at 12 months, probiotic supplementation resulted in overall transcriptional silencing by hypermethylation of regions related to immune functions. At 24 months, fewer or no biological terms were related to the differential DNA methylation patterns. Speculatively, our findings could indicate that effects of probiotic supplementation on epigenetic patterning and immunomodulation are most striking during the supplementation period. Allergy development was not clearly associated with differential methylation patterns, although we had limited statistical power for these comparisons.

However, additional studies on long term effects and different allergy outcomes in a larger cohort are required in order to further investigate these relationships in depth.

Zoonotic exposure to helminths and association with allergic sensitization in a Norwegian population

Nils Oskar Jõgi[1], Silver Peeter Siiak[1], Cecile Svanes[2], Randi Bertelsen[2] and William Horsnell[3]

Animal and human studies (from endemic regions) indicate that natural helminth infections are associated with protection from allergy. In Northern Europe natural helminth infections are less common than, for example, in sub-Saharan Africa. However, zoonotic exposure through direct and environmental contact with infected companion animals and urban carnivores, such as foxes, is still common. In contrast to natural exposure, zoonotic exposure to helminths, such as *Toxocara* spp., appear to be associated with increased risk of allergy. In this study we addressed prevalence of serum specific IgG4 antibody against helminths in a Norwegian two-generation cohort, as well as association with allergic sensitization. Serum levels of total IgG4, anti- *Toxocara* canis IgG4 and *Ascaris lumbricoides* IgG4 were established by ELISA in 2 cohorts recruited in Bergen, Norway: (1) parents born

KEY FINDINGS

Allergic sensitization was established by skin prick test reactivity to common inhalant allergens (one or more positive: 35%). Anti-*Ascaris* IgG4 was detected in 29% of the parents and 9% of the offspring. Anti-*Toxocara* IgG4 was detected in 17.5% of parents and 8.4% of offspring. Anti-T. canis IgG4 was signifcanlty higher in offspring exposed to cats or dogs (OR=5.54, 95% CI: 1.96; 15.74, p=0.001) a similar association was not found in the parent cohort. Univariate analyses of *Toxocara* seropositivity suggested an association with increased skin prick test reactivity to common inhalant allergens (OR=1.99[0.93-4.22]; p=0.07).

1945-1972 (n = 183) and (2) offspring born 1969 – 2003 (n = 263). Our preliminary findings show a high frequency of anti-helminth antibodies in a Norwegian cohort, associated with higher age or with having a pet cat or dog. Those with *Toxocara* seropositivity possibly had increased risk of allergic sensitization.

Early life priming for allergy by HDM allergen transfer through breast milk

Akila Rekima, Patricia Macchiaverni, Meri Tulic, Jon Genuneit, Alet Wijga, Karel Thijs, Nour Baiz, Isabella Annesi-Maesano, Naoki Shimojo, Susan Prescott and **Valérie Verhasselt**

House dust mite (HDM) is the main allergen source implicated in etiology of respiratory allergic disease. The lack of understanding on how allergy to HDM develops hampers successful prevention of allergy. In a mouse model where potential confounders are excluded, we demonstrated that oral exposure to Der p through breast milk strongly promotes allergic sensitization. **The aims was** to investigate whether early oral exposure to *Dermatophagoides Pteronyssinus* (*Der p) 1* major HDM allergen through breast milk modifies the risk of allergic sensitization and disease in children *Der p 1* was quantified by ELISA in milk from birth cohorts from France (EDEN), Australia (PRB and IFOS control arms), Japan (CHIBA) and The Netherlands (PIAMA). Allergic outcomes were assessed in children. In the latter 2 cohorts, Der p1 levels was also quantified in maternal and child environment. Oral exposure to HDM allergen through breast milk may contribute to the subsequent development of allergies in children. This original observation made in various environments settles the basis for innovative research on interventions aimed at inhibiting mother's milk HDM priming activities. This will guarantee allergy prevention by breast milk, the WHO's recommended food for children.

KEY FINDINGS

In the French and Australian cohorts, we found an increased risk of respiratory and food allergy in children breastfed by mothers with high levels of Der p1 in milk compared to low levels. Impact of HDM exposure through milk on atopic dermatitis in Japanese and respiratory allergies in Dutch cohorts are under analysis and preliminary results will be communicated at in-Flame meeting. These latter 2 cohorts analysis will also give informations on parameters governing Der p1 levels in breast milk.

Placenta histone acetylation in several immune regulatory genes is a potential predictor of allergy development

Hani Harb, Bilal Alashkar Alhamwe, Nathalie Acevedo, Johan Alm, Daniel P. Potaczek, Annika Scheynius and Harald Renz

Children born in families following anthroposophic lifestyle have been shown to be less susceptible to atopy development. It is known that epigenetic modifications underlain by environmental factors contribute to allergic predisposition. In the present study, we aimed to check if placental acetylation of H3 or H4 histones in promoters of several (potentially) allergy-related immune regulatory genes is associated with atopy development and if it is affected by the *anthroposophic lifestyle*. Placenta samples were obtained from 173 children born in anthroposophic, partial anthroposophic or non-anthroposophic families deriving from the *Assessment* of Lifestyle and *Allergic Disease During Infancy* (ALADDIN) prospective birth cohort study. Isolated placental cells were subjected to the chromatin immunoprecipitation (ChIP) assay assessing H3 or H4 histone acetylation in promoters of 9 genes, including *ASB2, HDAC4, IL13, IFNG, IL7R, IL10RA, CD14, SH2B3* and *FOXP3*. Atopic sensitization at 5 years of age was determined by a positive Phadiatop result (≥0.35 PAU/l). Our preliminary analysis seems to demonstrate the potential of placental histone acetylation assessment in several immune regulatory genes to predict the development of atopic sensitization later in life. If those epigenetic changes also functionally contribute to the development of allergy remains to be elucidated. At this early step, histone acetylation of placenta cells does not seem to be a major mechanism mediating anti-allergic effects of anthroposophic lifestyle, although further, more sophisticated analyses are required.

KEY FINDINGS

In the whole group, the presence of atopic sensitization at 5 years of age was associated with lower acetylation levels of H3 histone at *ASB2* or *IFNG* loci, which was independent of the lifestyle. Similar effects were observed in female children for *IL7R* and *IL13* promoters at H3 and *HDAC4* at H4 histones. In male offspring in turn, atopic sensitization was associated with increased H4 acetylation at *CD14* locus. Furthermore, H4 acetylation of *IFNG* was the highest in children born in anthroposophic and the lowest in those from non-anthroposophic families. In a female subgroup, *IFNG* H4 acetylation tended to be lower in the presence of atopic sensitization.

Longitudinal study of persistent organic pollutants in human milk: changes in lactation stages and effect on infant

Jian Du, Melvin C.L. Gay, Zoya Gridneva, Ching Tat Lai, Peter E. Hartmann, Robert D. Trengove, **Donna T. Geddes**

Persistent organic pollutants (POPs) are released into the environment and bio-accumulated in human adipose tissue via inhalation, ingestion or dermal adsorption. During milk synthesis, these bio-accumulated pesticides are secreted into the milk. As human milk is the main nutritional source for a breast-fed infant, monitoring of these POPs is essential to gauge the maternal environment, as raised levels of POPs will impact the full life span of the infant. This longitudinal study aimed to identify and quantify POPs in human milk over 12 months of lactation. Pre- and post- feed milk samples were collected from both breasts of 16 Western Australian (WA) mothers at 2, 5, 9 and 12 months postpartum. A miniaturized QuEChERs extraction optimized for human milk (1mL) was used, followed by quantitative GC-MS/MS analysis for 88 pesticides. Despite 4,4′-DDT being banned for several decades, its metabolite 4,4′-DDE was detected in most women's milk suggesting historical exposure while detection of 4,4′-DDT suggested exposure within the last decade or so. As lactation progressed, the level of POPs in milk decreased, suggesting that regular monitoring of human milk would provide an index of the levels of life-long exposure to POPs.

KEY FINDINGS

The average milk fat content was higher post-feed (Post: 55.0±22.7 g/L: Pre: (28.2±12.7 g/L; P< 0.001). Organochlorine pesticides (OCPs) such as 4,4′-DDE, 4,4′-DDT and beta-BHC were detected. 4,4′-DDE, a metabolite of 4,4′-DDT, was detected in 84% of samples. As 4,4′-DDE is lipophilic, the average concentration was higher in post-feed milk (Post: 1.8±1.3 µg/L; Pre: 1.15±1.05 µg/L). There was an overall significant decrease (P< 0.05) of 4,4′-DDE over 12 months. 4,4′-DDT was detected in 2 mothers (0.31±0.26 µg/L). Other classes of POPs such as OCPs, carbamate pesticide, pyrethroids and fungicides were not detected in any sample. Overall, POPs detected in milk samples of WA women were well below the tolerable limit.

IgE mediated food sensitisation and allergy in unselected rural and urban South African Toddlers

Maresa Botha, Wisdom Basera, Mike Levin, Claudia Gray

The prevalence of IgE-mediated food allergy in the South African population is unknown, however recently unexpectedly high level of sensitization have been seen in black African children with atopic dermatitis prompting this first population based prevalence study.This cross-sectional study recruited 12-36 month old toddlers from randomly selected crèches in Cape Town and communities in the rural district of Mqanduli. Parents of all eligible children completed a questionnaire and children had skin prick tests (SPT) for cow's milk, egg, soya, wheat, peanut, hazelnut and fish (cod). Participants with SPT test ≥1mm and not tolerant to that food had an oral food challenge to assess for IgE-mediated food allergy. 512 urban and 397 rural participants completed the study. There was significant difference in food allergy prevalence and all levels of food sensitisation between rural and urban participants. Children were most frequently sensitized to egg in both urban and rural cohorts (8.1%urban vs 3.0% rural) and all confirmed food allergies in the rural cohort were to eggs. Food allergy prevalence in urban Cape Town is comparable to that of other middle income countries, but significantly higher than in the rural communities from which migration occurs. These cohorts therefore provide the ideal opportunity for further investigation of protective factors still present in the rural environment.

Skin Prick test	Urban n=512 95%CI	Rural n=397 95%CI
≥1mm	**12.3%** 63 9.4-15.2	**4.5%** 18 2.1-6.0
≥3mm	**9.6%** 49 7.0-12.1	**2.5%** 10 0.9-4.0
≥7mm	**4.5%** 23 2.7-6.3	**1.5%** 6 0.3-2.7
OFC positive	**2.5%** 23 2.7-6.3	**0.5%** 2 0.2-1.2

Domestic pets and risk of IgE-mediated food allergy in infancy: findings from a cohort study.

John Molloy, Katrina Allen, Mimi LK Tang, Fiona Collier, Jennifer Koplin, John Carlin, Sarath Ranganathan, David Burgner, Richard Saffery, Anne-Louise Ponsonby, Peter Vuillermin on behalf of the BIS Investigator Group.

Cross-sectional evidence suggests that exposure to domestic pets may reduce the risk of food allergy. However, there are no prospective data regarding the relationship between pet ownership and challenge proven IgE-mediated food allergy in early life. Our objective was to investigate the association between pet ownership during pregnancy and offspring IgE-mediated food allergy. The Barwon infant study is a prospective birth cohort with antenatal recruitment (n=1074). Data regarding pet ownership (dog/cats/small animals) were collected via questionnaires completed by parents at 28 weeks pregnancy. At the 1 year review infants underwent skin prick testing to five foods, egg, peanut, cashew, cow's milk and sesame. Those with positive skin prick tests were offered an in-hospital open food challenge to determine their food allergy status. We provide strong prospective evidence that domestic pet ownership during pregnancy is associated with a decreased risk of IgE-mediated food allergy in offspring. Further studies are required to determine whether this association is mediated by microbial factors.

KEY FINDINGS

Among women who owned pets during pregnancy the rate of offspring food allergy was 34/576 (5.9%) compared to 27/207 (13.0%) among women without pets (OR 0.43, 95% CI 0.24-0.74). The majority of this association related to dog ownership (OR 0.41, 95% CI 0.24-0.73). Adjusting for the potential confounders, family history of allergy, ethnicity, socioeconomic status and number of siblings made less than 10% difference to the odds ratios.

Sensitizing capacity of raw and processed cow's milk in a murine sensitization model for food allergy

Anastriyani Yulviatun, Marcel Zwietering, Jeanne De Vries, Berber Vlieg-Boerstra

Recent studies suggest that exposure to a diversity of environmental microbes is beneficial to prevent non-communicable diseases (NCDs), including allergic disease. Our diet is one of the major contributors to this microbial exposure. The objective of this study was to evaluate and improve a previously developed FFQ (unpublished) to estimate dietary microbial exposure in Dutch young adults (age 19-31 years) as a possible novel determinant for the prevention of NCDs. Previously, a draft FFQ was developed by combining national food consumption data (1-day food diaries) with microbial contents of foods. Foods for the FFQ were selected on their contribution to microbial exposure level (MOM1) by stepwise regression analysis and to the variation in exposure based on estimated minimum, best and maximum levels (MOM2). The preliminary accuracy of our estimations was evaluated by comparing microbial estimates of 3-days food diaries of 34 adults with microbial analysis of their 1-day duplicate foods, taken on a different day. These preliminary results are the first step towards the evaluation of the FFQ. The next step will be to compare results from 3-days food diaries, with results based on the FFQ and microbial sampling of 24-h duplicate foods collected on the same day.

KEY FINDINGS

Food diaries: the exposure to aerobic spoilers varied between $4.1x10^3$ - $3x10^{10}$ CFU/day. Raw vegetables was the main food contributing to the level and variation of exposure. Lactic acid bacteria (LAB) varied between $5x10^6$ - $4.1x10^{11}$ CFU/day. Fermented dairy products, such as cheese and yoghurt, accounted for the major exposure and variation. Yeasts and moulds varied between $5x10^3$ - $5.1x10^9$ CFU/day with mould cheese and fresh fruits being the most relevant foods. *Duplicate food testing*: The ranges of microbial exposure were $2.5x10^5$ - $8.4x10^{10}$ CFU/day (aerobic spoilers), $<10^6$ - $1.9x10^{10}$

Exposure of the developing fetus and newborn to the mycotoxin deoxynivanelol

Astrid Hogenkamp, Prescilla Jeurink, Suzan Thijssen, Arash Alizadeh, Johanna Fink-Gremmels, Johan Garssen, Saskia Braber

Early life exposure to detrimental compounds can negatively impact development. We showed that the trichothecene deoxynivalenol (DON), a fungal metabolite found in grain-based human diets, acts as a disruptor of the intestinal tight junction network. As food antigens are transmitted through the placenta and breastmilk, we hypothesized that the developing fetus and/or newborn would be exposed to DON through these pathways, resulting in impaired gastro-intestinal development in the offspring. This, in turn, could lead to increased susceptibility to develop gastrointestinal disorders, such as food allergies. Upon arrival, C3H/HeOuJ mice were fed the control AIN93G diet and after two weeks of acclimatization, breeding pairs were formed. Females were either kept on the control diet or a control diet containing 10 mg DON/kg feed, until 15 days after delivery of the pups. After weaning, offspring were fed the control diet and were sensitized orally once a week for four weeks with OVA + CT. Acute allergic skin responses (ASR), shock symptoms, and specific plasma immunoglobulins were measured upon intradermal ovalbumin challenge. Our study suggests that maternal DON exposure in the current experimental setup does not affect the outcome of the OVA-specific food allergy in the offspring. It is possible that the DON-content of the diet was not sufficient to affect immune development in the offspring. Current research focuses on early life exposure of the developing fetus and/or newborn to different detrimental compounds in the maternal diet.

KEY FINDINGS

DON exposure led to increased intestinal permeability in the dams, as observed by increased translocation of FITC dextran across the intestinal interfaces. In the offspring there were no significant differences in the ASR. Flow cytometric analysis of the mesenteric lymph nodes and the spleen revealed no effect of maternal DON exposure on the effector responses in the offspring. OVA-specific and total immunoglobulin levels were similar between offspring of control dams and DON-treated dams

Influenza-induced memory T-cells confer protection over allergen-mediated acute airway inflammation

Chrysanthi Skevaki, Christoph Hudemann, Mikhail Matrosovich, Christian Möbs, Alessandro Sette, Stephan Becker, Harald Renz

The observation of an inverse relationship between infections and allergy has led to the hygiene hypothesis. Repeated upper viral respiratory tract infections have also been associated to less asthma and atopy development later in life. We aimed at validating this observation in a murine model of H1N1 HH/05/2009 infection and reveal mechanistic pathways at the level of adaptive immunity. In this context, we first characterized T memory (Tm) cell responses in the lungs and spleens of influenza infected animals with flow cytometry. We then observed an influenza-mediated protective effect over subsequent ovalbumin- or house dust mite-induced acute allergic airway inflammation as assessed by bronchoalveolar lavage cytology and cytokines, lung histology and airway hyperresponsiveness. CD8+ T effector memory (Tem) cell numbers were induced in animals exposed to both the virus and the allergen as compared to the allergen alone and the protective effect could be at least partially attributed to the presence of this cell population as shown by means of transfer experiments. Tem cells derived from influenza infected animals are defined by a unique cytokine production profile (IFNg, IL-10) in response to non-specific (anti-CD3/CD28) and virus peptides' cocktail stimuli. We conclude that cross-reactive Tm cellular responses are critical for modulating chronic inflammatory diseases and we are currently investigating whether our observations could be extrapolated to ubiquitous mechanisms underlying virus associated asthma, potentially amenable to therapeutic or preventive manipulations.

KEY FINDINGS

We identified homologies between virus- and allergen-derived predicted T-cell epitopes through extensive *in silico* work and assessed their relevance with *ex vivo* lymphocyte proliferation (^{3}T incorporation) and cytokine production (ELISPOT, ELISA) assays. As a proof-of-principle, we showed that immunization of the animals with a monovalent ovalbumin-free H1N1 commercially available vaccine conferred similar protection over experimental asthma.

Effects of lactobacilli on immune maturation in the intestinal mucosa

Sofia Nordlander, Dagbjört Petursdottir, Claudia Carvahlo Queiroz, Qazi Khaleda Rahman, Yeneneh Haileselassie, Marit Navis, Efthymia Kokkinou, Julia Henneman, Ivan Lio, Björn Brodin, Eva Sverremark Ekström

Early life microbial composition is important for immune maturation in the gut. We have previously shown in a cohort of Swedish children that the presence of certain species of *lactobacilli* in the intestine early in life is associated with reduced risk of allergic sensitization later in life, despite allergic heredity. We further wanted to investigate how the specific intestinal flora in the first weeks of life affected the development of the immune system. Stool samples collected from 2 week old children within the cohort were used to inoculate germ-free mice. The stool samples were pooled according to presence of certain *lactobacilli* as well as allergic heredity and known allergic sensitization of the individual children later in life. The immunological profile of the offspring of the inoculated mice was subsequently characterized. In order to assess mucosal immune responses, colonic lamina propria leukocytes were isolated and analyzed using fluorescence activated cell sorting (FACS).

KEY FINDINGS

We found that proportions of the CD4+ T-cell compartment differed in mice depending on the flora they had been exposed to. Frequencies and properties of FOXP3+ cells, indicative of regulatory T-cells, were altered. In addition, a population of RORgt+ cells was increased, suggesting induction of T helper 17 cells. Overall, the properties of gut flora with regards to content of *lactobacilli* and association with allergy had major effects on the development of the intestinal immune system.

A combination therapy of dietary galacto-oligosaccharides and budesonide in a house dust mite-model of asthma.

Kim Verheijden[1]; Saskia Braber[2]; Thea Leusink-Muis[1]; Suzan Thijssen[1]; Louis Boon[4]; Aletta Kraneveld[1]; Johan Garssen[1,3]; Gert Folkerts[1]; Linette Willemsen[1]

The standard treatment for airway inflammation in allergic asthma makes use of glucocorticosteroids, such as budesonide. Previously, we showed dietary non-digestible galacto-oligosaccharides (GOS) to suppress symptoms in a murine model for HDM-induced asthma. In this study we investigated the combined dietary GOS and budesonide treatment on allergic asthma in mice. BALB/c mice were sensitized and challenged with HDM while being fed a diet containing 0, 1 or 2.5 w/w% GOS. Budesonide (500ug/kg) was either or not instilled *oropharyngeally*. On day 14, airway resistance was determined and leukocyte subtypes were analyzed in the broncho-alveolar lavage fluid (BALF). Mast cell activation was assessed and chemokines and cytokines in lung homogenates and T-helper cell subtypes were phenotyped in lung cell suspensions by means of flow cytometry. HDM allergy increased airway responsiveness, budesonide treatment prevented this in mice fed the control or GOS diet. BALF leukocyte numbers were enhanced in HDM allergic mice. Budesonide treatment reduced the number of lymphocytes and eosinophils in HDM allergic mice, while the GOS diet reduced the number of eosinophils only. In mice fed GOS and treated with budesonide the lung inflammation, specifically eosinophilic infiltration, was largely abolished. Both GOS as well as budesonide reduced mast cell activation. The combination of GOS and budesonide, most effectively suppressed HDM induced increase in IL-33, CCL17, CCL22 and IL-13 in lung homogenates and the frequency of lung Th2 cells. Dietary intervention using GOS may be a novel way to further improve the effectiveness of anti-inflammatory drug therapy in asthma.

Our commitment to students and early career researchers:

Our early career researchers are fundamental to our network and make a major contribution to all of our meetings. A key outcome of our last meeting (Marburg 2015) was to establish a more formal **Early Career Researcher Network**. This is currently led by **Dr Daniel Munblit, an ECR)**, with mentorship from more senior members in particular **Prof Anita Kozyrskyj (Alberta).** They have been in regular email correspondence and Skype calls to discuss particular collaborative strategies, including crowdfunding, and the EMBA breast milk collaboration (See LactoActive outcomes and discussion on page 17).

Our network is also committed to supporting attendance of ECR at meetings through travel scholarships, which we have made available for the last two years, with help from our academic institutions and commercial sponsor Danone Nutricia.

Once again, one of the major highlights of our meeting was the career researcher 'rapid fire' bus-stop presentations. This was very stimulating and highly interactive, and gave the ECR opportunity to present their work. For some, it was the first opportunity to present results at an international meeting, and to develop collaborations and network with senior researchers. A number of important collaborative projects developed as a result. Another important discussion was progress with the in-FLAME **Mentoring Program**, and it was agreed that this is best and organic and informal process, are driven by the ECR but facilitated by more senior members as required. We hope that this will help provide additional career development and leadership opportunities for our junior members.

Report from our ECRs:

Some first actions:

- We agreed that mailing list is needed for easier communication (please let me know if anyone else should be added). You can also find a link to our ECR group **FaceBook page:** https://www.facebook.com/groups/973656822688679/

This is a closed group so feel free to share whatever you want but reserve SOP exchange and/or confidential data sets, for email communication. **Please join!**

Short and long term goals:

- Set up exchange programme (for short and long term visits (as a stand-alone project or as a part of their PhD/postDoc)) for ECRs. This will provide opportunities for new experience gain; bring ECR expertise to other teams; increase ECR profiles and give them a higher chance to be involved in a larger number of publications.
- We need to formulate:
  1. A list of major areas of interest/ideas for projects for each of us. This summary document will be circulated around all in-FLAME members then, so interested mentors can announce their interest.
  2. Propose topics for reviews of those topics that are not well covered at present.
  3. Assess the need in existing reviews update

Please be proactive and throw your ideas into the Facebook group, we may create dropbox later to make any future manuscript preparation easier.

- Please consider the possibility of using LactoActive BM metabolite study data for your personal grant applications, as Anita suggested.
- Aveni volunteered to assess maternal data across the cohorts, involved in LactoActive BM metabolite research

Breast Milk WUN in-FLAME/EMBA project

- We aim to prepare an outline of the project, to present it at EMBA meeting in one month time in Milan. Alongside we will start writing a proposal to Larsson-Rosenquist foundation (please see link) http://www.larsson-rosenquist.org/
- The basic concept is very well standardised longitudinal collection of breast milk samples from a number of milk banks (members of EMBA). Samples to be analysed for energy content, protein/fat content, immune active molecules, PUFAs, metabolomics, microbiome, vitamins. Some experimental work on reseeding processed BM with raw human milk. The number of samples to be analysed/number of centers involved/markers to measure is a matter of further discussion and primarily depends on funding.

We also aim to highlight existing gaps in BM research and produce a review paper on current knowledge on BM and health outcomes.

Sustainability and Strategy:

Our network is relatively young, and still developing its identity. Our broad remit was discussed over the course of the conference, including our role in both developing and promoting broad approaches to improving health, raising awareness of these concepts and in advocacy – in promoting the need for more holistic approaches to addressing global health challenges. This includes developing a public voice through blogs and social media – both as part of engaging in public conversations and sharing science in lay language. A number of immediate avenues for this were identified and will be capitalized on in the next 12 months. Several members have already started this process. This may eventually also lead to opportunity for crowd funding for specific initiatives (as proposed by our ECR Network).

Developing a position statement and publishing our 'perspective' will be another important dimension to articulating the public face of WUN in-FLAME. **Prof Susan Prescott** will lead a writing group on this roject in the coming months. Following the discussion of this meeting and the thoughts articulated in the Global Environment Working Group Proposal (Page 28) has further crystalised the broader vision of in-FLAME and how we can uniquely position ourselves in this area. This has also provided some immediate 'low hanging' fruit for people to add research questions to existing cohorts (such as nature relatedness questionnaires – page 29) which may also lead to comparative data between centres.

We do face a number of challenges and these were openly discussed. Although there is clear goodwill around collaboration the logistics can be challenging, because of the size of that the (very rapidly growing) network and diversity of interests, skills and assets (cohorts, repositories, technologies and platforms). However, our ultimate goal is to use this to our advantage. Ongoing opportunities to share our interests, activities and data are vital in developing and realizing our collaborative potential - and developing the relationships required to do this optimally. A clear awareness of 'who is doing what' and our capacities, is essential in face-to-face meetings. To this end there was strong consensus to continue to have annual workshops. There was strong support for our next workshop to be held in May 2017 in New York City, in parallel with the main WUN meeting.

Sharing our basic information through the network website is another facet of this. At present we do not have the resources for comprehensive information sharing, and this has been largely through simple spreadsheets that summarise our cohorts and expertise. It was agreed that this is still useful in the short term we plan to update this. Our spread sheets will be circulated after the meeting for members to update. **Prof Dianne Campbell (Sydney)** gave an update on the in-FLAME network website development at http://www.wuninflame.org. Which we hope will provide a forum for sharing documents, data and discussions. Passwords will be distributed to members.

A major Challenge for our network continues to be access to funding and resources (especially in developing collaborative platforms which can be time-consuming and expensive). As our profile develops and we begin to apply our expertise to new and novel collaborative ideas, we anticipate that new funding opportunities will arise. It is promising to see that we are already becoming proactive towards this.

In the coming weeks we will submit grant applications to support the next meeting. In the meantime we will also be exploring a number of avenues to support specific research projects – and ask that everybody explores new opportunities with us.

We look forward to welcoming you to New York next year – May 3-5th 2017.

A taste of other projects that are developed (since 2015) as part of *in-FLAME* collaborations

In addition to the many new projects formally presented there were an even larger number that developed organically during the meeting as a result of the new relationships formed. This summary captures a number of these – based on what members have reported back so far.

Project	Who	What
GENERAL BIOINFORMATICS SUPPORT	Fiona Brinkman	Expertise available to those who are interested - bioinformatics and data integration for inflammatory disease/innate immunity/allergy and asthma, with data integration projects involving the Canadian AllerGen NCE ***Please feel free to contact Fiona if you need assistance***
Impact of oligosaccharides supplementation on breast milk mediated prevention of allergy	Valérie Verhasselt	A collaborative project with Johan Garssen's team on impact of oligosaccharides on oral tolerance induction through breast milk (following Cape Town meeting). Will include both immune as well as physiological measurements in offspring animals focussing on gut and lung function.
Plans for new breast milk collaborations on mother-child cohorts	Valérie Verhasselt	Working with cohorts run by Carel Thijs (Maastricht, Holland) Naoki Shimojo (Japan) Debbie Palmer (Australia) Maria Jenmalm (Sweden)
Allergy risk factors in breast milk	Valérie Verhasselt	Mechanistic studies in collaboration with: Clarissa Prazeres da Costa (Germany) (Marburg) Harald Renz and Hani Harb (Cape Town- Marburg)
LPS variants in ALADDIN	Annika Scheynius Susanne Brix Pedersen	During the meeting a new collaboration was established with, Copenhagen, Denmark, to analyze fecal samples collected at early time points in the birth cohort ALADDIN (see attachment). A special focus is to investigate the different variants of LPS (as Susanne presented) in relation to life style and allergic outcome in the children.
Epigenetics in the placenta	Annika Scheynius Harald Renz	A pilot study was initiated with Harald Renz, Marburg, to investigate possible epigenetic differences in the placenta specimens collected in ALADDIN (see attachment). Based on the results from the pilot study indicating a difference for several genes associated to lifestyle of the mother we decided at the meeting in Freiburg to expand the analyses with additional 128 placenta samples.
New microbiome collaorations in Sweden	Annika Scheynius Christina West and Maria Jenmalm	Planning for a new microbiome study and where the ALADDIN cohort might be included.
Autoimmune studies (1)	Aveni Haynes Chrysanthi Skevaki	Exploring idea of doing a study using existing cohorts to investigate the association between risk factors and inflammatory markers with the development of autoantibodies in childhood (Type 1 diabetes, Coeliac disease, Hypothyroidism, Juvenile Arthritis etc)
Autoimmune studies (2)	Aveni Haynes Jon Genuneit Cathy Thornton	Develop study ideas using Jon's cohorts to investigate the association between maternal HbA1C in mother's with gestational diabetes and inflammatory/metabolic markers in the offspring

Project	Who	What
Gestational Diabetes	Aveni Haynes Susan Prescott Rae Chi Huang	Investigate the programming effect of GDM in the ORIGINs cohort perhaps? In particular, are there programming effects of GDM per se which are independent to BMI and excessive gestational weight gain? Or can these be modified by exercise in women with GDM etc
Food Allergy (1)	Aveni Haynes Katie Allen Jennifer Koplin	Ongoing discussions on potential collaboration
Food Allergy (2)	Daniel Munblit Diego Peroni (Naoki Shimojo Alexander Pampura Audrey Dunn Galvin	Plans of collaboration on food allergy QoL questionnaires validation in Russia, Italy and Japan with subsequent study on FA children QoL (UK, Russia, Japan, Italy, Ireland)
FADS collaboration	Berthold Koletzko Karen Simmer Suzanne Meldrum Rae Chi Huang Susan Prescott	This is an ongoing collaboration exploring FADS gene analysis in relation to the PUFA studies (Nina di Vaz, Susan Prescott). The results will be analysed in relation to • allergy • neurodevelopment • metabolic measures on these cohorts (Several manuscripts anticipated) Berthold Koletzko's group in Munich have also performed metabolomic analyses of the blood samples of Western Australian subjects. Currently Sebastian Rauschert is in Perth for a 6 months research fellowship to collaborate with the team at the **Telethon Kids Insitute** to analyse the data and prepare the publications (supported by the EU FP7 EarlyNutrition Project).
Fatty acids (2)	Carel Thijs Others in metabolism group	-replication of results of immune components and fatty acids in breast milk in existing cohorts and extension to new hypotheses and new biomarkers
BIRTH consortium	Multiple members (see EU 2020 information)	- submission of application to Horizon 2020 grant with Daniel Munblit as coordinator: collaboration initiated in Milan, made concrete in Cape Town - currently searching for other potential grant proposals (e.g. Newton-Al Farabi foundation) - Planning fundraising for non-communicable diseases research
Breast milk and appetite	Donna Geddes	Breast milk appetite hormones across the first 12 months of lactation and their relationship with maternal and infant body composition (measured by BMI, bioimpedance and ultrasound skin folds). Ability to run large sample sets for fat, protein, lactose, lactoferrin, lysozyme, BBSL, ALP, Na, K with very small amounts of milk. Appetite hormones are also being currently worked up (leptin in whole milk, adiponection, grehlin and looking at resistin, obestatin.
POPs in breast milk	Donna Geddes With potential to collaborate with Canadian and Noregian members	Identification of pollutants in breastmilk: Validation of a sensitive technique requiring only 1mL of milk. Cross sectional cohort (n=50, Perth, 2, 5, 9, 12 months lactation), longitudinal cohort (n=15, Perth, 2, 5, 9, 12 months lactation), 24 hour milk profiles that allow accurate calculation of dose rather than an estimation.

Project	Who	What
Review on the importance of natural environments and biodiversity in NCDs	Alan Logan Susan Prescott Jeff Craig	Will review evidence that is emerging on 'green space' and early outcomes, including evidence that the benefits may begin before birth. Hope to also explore new projects on this.
Peruvian breast milk analysis	Donna Geddes	We are poised to run 21 micronutrients in milk hopefully within a month on a large sample set from Peru (>500 samples).
Pollutants and mental health in children	Felice Jacka Merete Eggesbø	Will be meeting in Oslo in Sept/October to begin planning for collaborative projects examining the possible relationship of early life exposure to pollutants and mental health outcomes in children. We hope to utilise data from more than one cohort study.
Diet and inflammation	Felice Jacka Susanne Brix Pedersen	We hope to collaborate on a project examining dietary exposures and their relationship to inflammatory markers and gut microbiota, as well as child outcomes in COPSAC cohort study. Susanne will also advise us on the analysis of some of the Australian data.
Nutrition and 'Leaky gut' hypothesis	Felice Jacka Meri Tulic	Preliminary plans to share mucosal biopsy data with to examine 'leaky gut' hypotheses (using Ussing Chambers). Pending funding (currently submitted).
Biosensor for detecting allergic risk in newborns	Vicki Clifton	Has been developing and continue to develop the biosensor for detecting allergic risk in newborns. This is a collaboration with Tanya Monro, Peter Hoffmann, Andrew Tai. It is currently a PhD project conducted by Nurul Zainal. This was initiated from attendance at the Washington In-FLAME meeting
Thymic Studies on various cohorts	Ralph Nanan Peter Vuillermin Dianne Campbell Anita Korzyrskyj	Collaboration to analyse thymic size in relationship to outcomes and exposures in various cohorts (including CHILD (Canada), Barwon Infant Study (Melbourne) and ORIGINS (Perth) and others.
BOPIA (Peanut and SCFA)	Dianne Campbell Paul Turner David Fleisher Anita Kozyrskyj Naoki Shimojo Shuichi Suzuki	Collaborations with Imperial college, Denver, Alberta/ Edmonton, Chiba (see main report)
Effects of vitamin D on Treg in protection from atopic dermatitis	Clarissa de Costa Ralph Nanan Pete Vuillermin	Potential collaboration to examine vitamin D effects on Treg protection against allergy and function in AD.
Effects of labour and delivery method in immune phenotypes	Jon Genuneit Peter Vuillermin Susanne Brix Bianca Schaub	Proposed collaboration to investigate the effects of duration of labour and of delivery mode on immune phenotypes in cord blood in 5 cohorts from Copenhagen, Munich, Melbourne, Ulm. The first teleconference planned for end of 07/2015. This will build on finding in BIS suggesting that a relationship between nTreg and FA is modified by exposure to labour.
New allergens, raw milk research program (see main report)	Johan Garssen John Sinn.	Idea initiated in Cape Town. Funding for 1 PhD student who will focus on raw milk and its tolerance inducing capacity. Besides allergens/antigens we will focus on milk enzymes such as alkaline phosphatase which might be highly relevant in inflammation management.
SCFA in sperm and success of IVF (in vitro fertilisation)	Johan Garssen Philip Calder	Sperm contains unique non-digestible oligosaccharides that are very similar to those in breastmilk. During the coming year we will analyse non-digestible oligosaccahrides in sperm samples and might link this to success of the IVF therapy.

Project	Who	What
'Natural' toxins in foods	Johan Garssen Jon Genuneit	As discussed during this Marburg meeting and in Cape Town we will focus on natural toxins present in foods/cereals/rice etc. such as mycotoxins (DON). In preclinical studies we discovered that low levels of mycotoxins (levels that are allowed according to international safety guidelines) can serve as adjuvant for food allergens. In Asia, especially China mycotoxin levels in milk and food and even breast milk are relative high and might be responsible at least in part for the increase in incidence/severity of allergies. Together with Jon Genuneit we will analyse breastmilk samples for mycotoxins/DON. Using food questionairres we are already estimating the dosage consumed in Asia.
Collaborative Animal models (SCFA and fibre)	Johan Garssen Marie Bodinier	Marie is running almost similar studies with pregnant/lactating animals as they do at Utrecht University. Will exchange samples (stool/blood/breastmilk) for collaborative experiments.
Suggestion: that we make a coordinated international appeal to preserve the assets that we have around the world: for further discussion	Tim Takaro	Suggested that WHO/other entities may be interested in keeping alive existing cohorts with deep exposure assessment, genetics, epigenetics, diet, microbiome, psych-soc. assessment and multiple biological samples? I know of at least one (CHILD) where our babies are reaching the 5 year clinical endpoints without funding to analyze samples or keep the cohort together past five. New cohorts can be very interesting, but It makes a lot of sense to keep previous investments alive too!
Comment:	Katie Allen	I was at WHO after the meeting and they are looking to start a range of pre-pregnancy cohorts to look at an "early healthy start to life" around the world. I suggested speaking to our inFLAME group would be valuable! It is having networks like ours that bring knowhow together which i think are really valuable. It means we can be ready when opportunities arise.
Vitamin D in Africa (similar protocol to Australian study)	Rose Kamenwa Debbie Palmer Kristina Rueter Susan Prescott Mike Levin	In this double blind placebo controlled randomised trial 120 infants with a family history of allergic disease receive 400 IU vitamin D supplementation/day or placebo for 6 months. In this study a UV dosimeter will also be worn by the infants from birth to 6 months of age to measure actual UVB exposure.
BENEFIT RCT (whole foods approach to allergy prevention)	Debbie Palmer Kristina Rueter Dianne Campbell Susan Prescott Daniell Munblitt	This study aims to look at the influence of a mixed 'whole food' diet delivering allergenic foods in the context of immunomodulatory nutritents (PUFA, prebiotics, vitamin D etc in whole foods). After a fruitful discussion we came to the agreement that this study should be limited to a maternal dietary intervention only (from 20 weeks of gestation until cessation of breastfeeding). Apart from nuts, egg and fish the diet will also include balsamic vinegar. Groups will be divided into 'high consumption" of thesefoods versus "low consumption" (specific amount still needs to be determined).Discussions are ongoing and other collaborators are welcome.

Project	Who	What
Pet ownership and endotoxin variants	Peter Vuillermin Susanne Brix	Will investigate the hypothesis that the protective effect of pet ownership on allergic outcomes is mediated by enteric colonisation of the mother and/or infant gut with penta-acetylated endotoxin producing bacteria. This will add substantial value to the NHMRC Pete was awarded last year for 16s and SCFA measures in BIS.
Maternal diet, microbiome and thymic size	Peter Vuillermin Ralph Nanan	Plan to investigate the hypotheses that a maternal gut microbiome that produces lower levels of SCFAs during pregnancy is associated with (a) pre-eclampsia, and (b) reduced thymic size and nTreg in the offspring. Again, adding substantial value to funded work underway in BIS.
Mechanisms of early oral tolerance – sharing samples and data from infant feeding RCT	Peter Hsu Debbie Palmer Dianne Campbell Susan Prescott	Treg studies and other collaborative analyses on samples from infant feeding studies (especially STAR, STEP. BEAT and QUEST). Will form a new partnership between Telethon Kids Institute (Perth) and Kids Research Institute (Sydney). Opportunities for PhD student exchange. We hope to link this with the EU project (iFAAM) lead by Clare Mills.
Larger scale comparison on POPs and other pollutants in in-FLAME cohorts – in relation to specific outcomes	Merete Eggesbø Chrysanthi Skevaki (Amanda Wheeler, Donna Geddes, Tim Takaro TBC)	Initiative to explore future use *in-FLAME* cohorts for a combined analysis within immunology, the details not yet decided on.
Interactions between POPs and the microbiome	Shuichi Suzuki John Penders Koen Venema	Shuichi had some interesting ideas about investigating the link between the microbiome and the metabolism/bioavailabilty of ingested pollutants and I thought there might be some possibilities to examine this within the TIM-system. Currently being explored further. Recommend also including Merete Eggesbø in the discussions.

In-FLAME Publications:

2016 – accepted or now published (*original research)

1. AC Logan, FN Jacka, SL Prescott, Immune-Microbiota Interactions: Dysbiosis as a Global Health. Current Allergy Asthma Reports Curr Allergy Asthma Rep. 2016 Jan;16(2):13

2. AC Logan, SL Prescott, JC Craig. Journal of Physiological Anthropology. Natural Environments, Nature Relatedness and the Ecological Theatre: Connecting Satellites and Sequencing to Shinrin-yoku J Physiol Anthropol. 2016 Jan 13;35(1):1. doi: 10.1186/s40101-016-0083-9.

3. West CE, Jenmalm MC, Kozyrskyj AL, Prescott SL.Probiotics for treatment and primary prevention of allergic diseases and asthma: looking back and moving forward. Expert Rev Clin Immunol. 2016 Mar 4:1-15. [Epub ahead of print]

4. *H Harb, M Amarasekera, S Ashley[3] MK. Tulic, PI Pfefferle, DP. Potaczek, H Renz, D Martino, DA. Kesper and SL. Prescott. Epigenetic regulation in early childhood: a miniaturized andvalidated method to assess histone acetylation. International Archives of Allergy and Immunology, Int Arch Allergy Immunol. 2015;168(3):173-81. doi: 10.1159/000442158. Epub 2016 Jan 21

2016 – submitted or under revision

5. *H Harb, M Amarasekera, S Ashley[3] MK. Tulic, PI Pfefferle, DP. Potaczek, H Renz, D Martino, DA. Kesper and SL. Prescott. Folate status as a modifier of epigenetic profile in human neonatal $CD4^+$ T cells. Submitted

6. *H Harb, M Amarasekera, J Irvine, C Hii, DA Kesper, Y Ma, N D'Vaz, A Ferrante, H Renz, SL Prescott. Neonatal PKCζ drives T-cell maturation and is epigenetically modified by maternal fish oil intake. Allergy (submitted March 2016)

7. Van den Berg J, Skevaki C, Jones N….Thornton CA for the InFLAME Network. Immune biomarkers in the spectrum of childhood non-communicable diseases. J Allergy Clin Immunol (Outline has been accepted by JACI – now in preparation, as per report by Metabolism Working Group).

8. MC Jenmalm, CE West, SL Prescott, AL Kozyrskij, A 'Probiotics in allergy prevention: time to revisit recommendations?' Clin Exp Allergy (in preparation)

9. MC Jenmalm, CE West, SL Prescott, AL Kozyrskij, The use of probiotics for the prevention and treatment allergic diseases and asthma' Expert Review of Clinical Immunology (in preparation)

10. P Vuillermin, J Genuneit, C West, D Campbell, K Allen, SL Prescott et al, The potential link between dietary intake of fermentable fibre, the production of short chain fatty acids by gut microbiota and asthma and allergic disease. In preparation.

11. *Nour Baiz, Patricia Macchiaverni, Meri Tulic, Akila Rekima, Antonio Condino Neto, Isabella Annesi-Maesano* and V. Verhasselt*: respiratory allergen in human breast milk are risk factor for allergy (in preparation)

12. *Meri Tulic, Akila Rekima, Jon Genuneit, Christelle Bonnart, Nathalie Vergnolles, Hani Harb, Samara Medeiros, Samantha Zanelli, Harald Renz, Susan Prescott and Valérie Verhasselt: protease mediated priming of food allergy in early life by house dust mite allergen (in preparation)

13. Valérie Verhasselt, Philip Calder and Daniel Munblit : new insights on breast milk long term health beneficial effects (in preparation – authors order, contributors and exact title not yet finalized)

14. Munblit D, Sheth S, Abrol P, Treneva M, Peroni DG, Chow L-Y, Boner AL, Pampura A, Warner JO, Boyle RJ Exposures influencing total IgA level in colostrum. J DOHaD (under revision)

15. *Munblit D, Treneva M, Peroni DG, Colicino S, Chow L-Y, Dissanayeke S, Pampura A, Boner AL, Boyle RJ and Warner JO. Colostrum and breast milk of mothers from London, Moscow and Verona: determinants of growth factor levels and cytokines detectability. European Journal of Nutrition (In preparation)

16. Munblit D, Treneva M, Peroni DG, Colicino S, Chow L-Y, Dissanayeke S, Pampura A, Boner AL, Boyle RJ and Warner JO Colostrum immune composition and immunological outcomes assessment using Principal Component analysis (PCA). (*In preparation*).

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17. * Tulic MK, Vivinus-Nébot M, Rekima A, Rabelo Medeiros S, Bonnart C, Shi H, Walker A, Dainese R, Boyer J, Vergnolle N, Piche T, Verhasselt V. Presence of commensal house dust mite allergen in human gastrointestinal tract: a potential contributor to intestinal barrier dysfunction. Gut. 2015 Dec 8. pii: gutjnl-2015-310523.

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25. K Rueter, SL Prescott and J Palmer. Nutritional approaches for the primary prevention of allergic disease: an update, Journal Paediatrics and Child Health, J Paediatr Child Health. 2015 Jul 2. [Epub ahead of print]

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28. SL Prescott, "Origins: Early Life Solutions to the Modern Health Crisis" First published in 2015 by UWA Publishing Crawley, Western Australia 6009, Copyright © Susan L. Prescott (ISBN 978-1-74258-670-0 (based on the philosophies of the WUN in-FLAME network)

29. K Rueter , A Haynes, SL Prescott. Developing primary prevention strategies to prevent allergic disease, Curr Allergy Asthma Rep. 2015 Jul;15(7):40.

30. *Mathilde Turfkruyer, Akila Rekima, Patricia Macchiaverni, laura Le Bourhis, Gijs Van den Brink, Vanesa Duncan, Meri Tulic and Valérie Verhasselt. Allergy prevention by oral tolerance is inefficient in neonates due to physiological vitamin A deficiency. Mucosal Immunology (on-line November 2015)

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