Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates

Preterm neonates are susceptible to gastrointestinal disorders such as necrotizing enterocolitis (NEC). Maternal milk and colostrum protects against NEC via growth promoting, immunomodulatory, and antimicrobial factors. The fetal enteral diet amniotic fluid (AF), contains similar components, and we hypothesized that postnatal AF administration reduces inflammatory responses and NEC in preterm neonates. Preterm pigs (92% gestation) were delivered by caesarean section and fed parental nutrition (2 days) followed by enteral (2 days) porcine colostrum (COLOS, n = 7), infant formula (FORM, n = 13), or AF supplied before and after introduction of formula (AF, n = 10) in experiment 1, and supplied only during the enteral feeding period in experiment 2 (FORM, n = 16; AF, n = 14). The NEC score was reduced in both AF and COLOS pigs, relative to FORM, when AF was provided prior to full enteral feeding (9.9 and 7.7 compared with 17.3, P <0.05). There was no effect of AF when provided only during enteral feeding. AF pigs showed decreased bacterial abundance in colon and intestinal inflammation-related genes (e.g., TNF-α, IL-1α, IL-6, NOS) were downregulated, relative to FORM pigs with NEC. Anti-inflammatory properties of AF were supported by delayed maturation and decreased TNF-α production in murine dendritic cells, as well as increased proliferation and migration, and downregulation of IL-6 expression in intestinal cells (IEC-6, IPEC-J2). Like colostrum, AF may reduce NEC development in preterm neonates by suppressing the proinflammatory responses to enteral formula feeding and gut colonization when provided before the onset of NEC.
Establishment of tolerance to commensal bacteria requires a complex microbiota and is accompanied by decreased intestinal chemokine expression

Intricate regulation of tolerance to the intestinal commensal microbiota acquired at birth is critical. We hypothesized that epithelial cell tolerance toward early gram-positive and gram-negative colonizing bacteria is established immediately after birth, as has previously been shown for endotoxin. Gene expression in the intestine of mouse pups born to dams that were either colonized with a conventional microbiota or monocolonized (Lactobacillus acidophilus or Eschericia coli) or germ free was examined on day 1 and day 6 after birth. Intestinal epithelial cells from all groups of pups were stimulated ex vivo with L. acidophilus and E. coli to assess tolerance establishment. Intestine from pups exposed to a conventional microbiota displayed lower expression of Ccl2, Ccl3, Cxcl1, Cxcl2, and Tslp than germ-free mice, whereas genes encoding proteins in Toll-like receptor signaling pathways and cytokines were upregulated. When comparing pups on day 1 and day 6 after birth, a specific change in gene expression pattern was evident in all groups of mice. Tolerance to ex vivo stimulation with E. coli was only established in conventional animals. Colonization of the intestine was reflected in the spleen displaying downregulation of Cxc12 compared with germ-free animals on day 1 after birth. Colonization reduced the expression of genes involved in antigen presentation in the intestine-draining mesenteric lymph nodes, but not in the popliteal lymph nodes, as evidenced by gene expression on day 23 after birth. We propose that microbial detection systems in the intestine are upregulated by colonization with a diverse microbiota, whereas expression of proinflammatory chemokines is reduced to avoid excess recruitment of immune cells to the maturing intestine.

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Nature of bacterial colonization influences transcription of mucin genes in mice during the first week of life

In summary, our data show that development of the expression of genes encoding secreted (Muc2/Tff3) and membrane-bound (Muc1/Muc3/Muc4) mucus regulatory proteins, respectively, is distinct and that the onset of this development may be accelerated by specific groups of bacteria present or absent at the mucosal site.

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Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Bahl, M. I. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
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TLR2 Controls Intestinal Carcinogen Detoxication by CYP1A1.
Intestinal cytochrome P450 subclass 1A1 (CYP1A1) contributes to a metabolic "shield" protecting the host from ingested carcinogens such as polycyclic aromatic hydrocarbons (PAH). The expression of CYP1 (including CYP1A2 and CYP1B1) is considered to depend solely on a heterodimeric transcription factor consisting of the arylhydrocarbon receptor (AHR) and the AHR nuclear translocator (ARNT). So far, no interference has been noted between the regulation of CYP1 and the activation of Toll-like receptor 2 (TLR2), which modulates the inflammatory response to bacterial cell wall components in immune cells and enterocytes. Here we report that intestinal CYP1A1 is silenced in TLR2-deficient mice, even when under exposure to the carcinogenic PAH benzo[a]pyrene (BaP). In contrast, hepatic CYP1A1 was moderately induced in TLR2-deficient mice without restoring their ability to clear BaP from systemic circulation, as present in wild-type animals. After feeding of BaP for 21 days, only TLR2(-/-) mice, but not their wild type littermates developed polyps in the colon. Gene expressions and protein concentrations of AHR and ARNT in the intestine did not differ between the genotypes. In conclusion, the presence of ligands for TLR2 of bacterial origin seems to be crucial for detoxication of luminal carcinogens by CYP1A1 in the intestine. This unprecedented finding indicates a complex interplay between the immune system of the host and intestinal bacteria with detoxication mechanisms. This highlights the relevance of intestinal microbiota when trying to unravel pathways present in mammals and opens new perspectives for research in human health.

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Authors: Do, K. (Intern), Fink, L. N. (Intern), Jensen, T. E. (Forskerdatabase), Gautier, L. (Intern), Parlesak, A. (Intern)
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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.545 SNIP 1.141 CiteScore 3.54
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.74 SNIP 1.147 CiteScore 3.94
ISI indexed (2013): ISI indexed yes
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Scopus rating (2012): SJR 1.945 SNIP 1.142 CiteScore 4.15
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ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
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Bovine colostrum is superior to enriched formulas in stimulating intestinal function and necrotising enterocolitis resistance in preterm pigs

Milk contains immunomodulatory compounds that may be important to protect the immature intestine in preterm neonates from harmful inflammatory reactions involved in disorders like necrotising enterocolitis (NEC). We hypothesised that bovine colostrum and milk formulas enriched with sialic acids (SL), gangliosides (Gang) or osteopontin (OPN) would improve gastrointestinal function and NEC resistance in preterm neonates. Forty-seven caesarean-delivered preterm pigs were given total parenteral nutrition for 2 d followed by 1.5 d of enteral feeding. In Expt 1, a control formula was compared with an OPN-enriched formula (n 13), while Expt 2 compared a control formula with bovine colostrum or formulas enriched with Gang or SL (n 4-6). OPN enrichment decreased NEC severity relative to control formula (P
Adherent-invasive Escherichia coli (AIEC) are reported to inhabit the gut mucosa in Crohn’s disease (CD), however, little is known about the importance of host factors for the interplay between AIEC and the human gut. To examine if differences in bacterial adhesion patterns are disease associated, the AIEC-prototype strain LF82 was evaluated for its ability to adhere to ileal and colonic biopsies from CD and healthy controls (HC). Moreover, the efficacy of the non-pathogenic E. coli Nissle 1917 (ECN) in averting LF82 adhesion to ileal mucosa was assessed. Similar numbers of LF82 adhered to ileal and colonic biopsies from CD and HC. A significantly greater LF82 attachment to ileal versus colonic mucosa was found in HC (P < 0.01), however, not in CD. ECN did not reduce the adhesion of LF82 to ileal specimens in CD or HC. These results show that enhanced bacterial adhesion ability is unlikely to play any significant role in CD, thus implying that other host protective factors may be impaired in CD. Further, exclusion of LF82 attachment by ECN co-incubation does not appear to represent a relevant treatment regimen.
General information
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Organisations: Center for Biological Sequence Analysis, Department of Systems Biology, Copenhagen University Hospital
Authors: Jensen, S. R. (Intern), Fink, L. N. (Intern), Nielsen, O. H. (Ekstern), Brynskov, J. (Ekstern), Pedersen, S. B. (Intern)
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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.928 SNIP 0.749 CiteScore 1.89
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Web of Science (2013): Indexed yes
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Scopus rating (2012): SJR 0.899 SNIP 0.696 CiteScore 2.01
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BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.87 SNIP 0.706 CiteScore 2.09
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.097 SNIP 0.678
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Scopus rating (2009): SJR 1.098 SNIP 0.804
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.015 SNIP 0.687
Scopus rating (2007): SJR 1.022 SNIP 0.683
Scopus rating (2006): SJR 1.083 SNIP 0.809
Scopus rating (2005): SJR 1.066 SNIP 0.725
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.909 SNIP 0.61
Scopus rating (2003): SJR 0.87 SNIP 0.617
Scopus rating (2002): SJR 0.928 SNIP 0.596
Scopus rating (2001): SJR 0.832 SNIP 0.585
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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.362 SNIP 1.551 CiteScore 4.14
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.879 SNIP 1.782 CiteScore 4.38
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.131 SNIP 1.582 CiteScore 3.77
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.659 SNIP 1.494 CiteScore 3.16
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.504 SNIP 1.485
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.587 SNIP 1.44
Web of Science (2009): Indexed yes
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Scopus rating (2008): SJR 1.861 SNIP 1.254
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Scopus rating (2005): SJR 0.953 SNIP 1.033
Scopus rating (2004): SJR 1.167 SNIP 1.331
Scopus rating (2003): SJR 1.232 SNIP 1.244
Scopus rating (2002): SJR 1.092 SNIP 1.129
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Ileal adhesion of virulent E. coli LF82 is not enhanced in Crohn’s disease

Adherent-invasive Escherichia coli (AIEC) comprise a new group of E. coli species named from their distinctive ability to adhere to and invade the intestinal epithelium. The AIEC strains have been associated to the ileal mucosa in Crohn’s disease (CD), and the impact of AIEC in the pathogenesis of CD has been further strengthened from the evidence that the ileum in CD harbors an abnormally high number of E. coli species. The aim of this study was to examine the adhesion of the AIEC reference strain, LF82, to tissue samples from ileum and colon in CD and healthy controls. A second purpose was to assess the probiotic efficacy of E. coli Nissle 1917 (ECN) in averting LF82 adhesion to ileal mucosa. Ileal and colonic specimens were obtained from patients with CD ileitis and controls (n=10). A model was developed to investigate bacterial adhesion to intestinal biopsies and comprised: 1) incubation of tissue (inclusive of mucous) with 10^7 bacteria or buffer for 1 hour, 2) removal of non-adhered bacteria by extensive washing, and 3) absolute quantification of tissue-adhered LF82 and indigenous E. coli by a pre-validated assay including quantitative real-time PCR. Selective primers- and probes were designed specifically for targeting the pMT1-like plasmid in LF82 and E. coli 16S ribosomal DNA for quantifying the general E. coli population. Bacterial numbers were related to tissue weight. A thoroughly validated model with a coefficient of variation <2 % was developed and employed for investigation of the bacterial adherence to human intestinal specimens. LF82 adhered to intestinal biopsies in both CD and controls. Enhanced adhesion was, however, not observed in the ileum as compared to the colon in CD, which was in contradiction to controls that had a significantly higher LF82-attachment to the ileal epithelium as compared to that of the colon (P <0.01). The variation in LF82 adhesion between ileal and colonic specimens was more prominent in CD than in controls. Although not statistically significant, a trend towards higher counts of indigenous E. coli was observed in the ileum as compared to the colon of CD, and the number of indigenous LF82 and total E. coli bacteria tended to be inversely correlated in both ileum and colon tissue. Further, ECN did not avert the adhesion of LF82 to ileal specimens, but instead ECN likely favoured LF82 adhesion particularly in CD. ECN did also adhere to the ileal mucosa. Conclusively it was shown that LF82 preferentially adhere to ileal tissue in controls, but not in CD suggesting that the intestinal microbiome of the colon is changed in terminal ileitis. Co-incubation with ECN tended to increase ileal LF82 adhesion, thus highlighting that careful mechanistic studies are warranted before including ECN in clinical studies. The current study demonstrates a great variability in host LF82 interactions within the group of patients with CD ileitis, thus stressing individual response patterns against LF82.
Role of Natural Killer and Dendritic Cell Crosstalk in Immunomodulation by Commensal Bacteria Probiotics

A cooperative dialogue between natural killer (NK) cells and dendritic cells (DCs) has been elucidated in the last years. They help each other to acquire their complete functions, both in the periphery and in the secondary lymphoid organs. Thus, NK cells' activation by dendritic cells allows the killing of transformed or infected cells in the periphery but may also be important for the generation of adaptive immunity. Indeed, it has been shown that NK cells may play a key role in polarizing a Th1 response upon interaction with DCs exposed to microbial products. This regulatory role of DC/NK cross-talk is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with commensal bacteria such as lactic acid bacteria (LAB). We here review NK/DC interactions in the presence of gut-derived commensal bacteria and their role in bacterial strain-dependent immunomodulatory effects. We particularly aim to highlight the ability of distinct species of commensal bacterial probiotics to differently affect the outcome of DC/NK cross-talk and consequently to differently influence the polarization of the adaptive immune response.
Bifidobacterium bifidum Actively Changes the Gene Expression Profile Induced by Lactobacillus acidophilus in Murine Dendritic Cells

Dendritic cells (DC) play a pivotal regulatory role in activation of both the innate as well as the adaptive immune system by responding to environmental microorganisms. We have previously shown that Lactobacillus acidophilus induces a strong production of the pro-inflammatory and Th1 polarizing cytokine IL-12 in DC, whereas bifidobacteria do not induce IL-12 but inhibit the IL-12 production induced by lactobacilli. In the present study, genome-wide microarrays were used to investigate the gene expression pattern of murine DC stimulated with Lactobacillus acidophilus NCFM and Bifidobacterium bifidum Z9. L. acidophilus NCFM strongly induced expression of interferon (IFN)-beta, other virus defence genes, and cytokine and chemokine genes related to the innate and the adaptive immune response. By contrast, B. bifidum Z9 up-regulated genes encoding cytokines and chemokines related to the innate immune response. Moreover, B. bifidum Z9 inhibited the expression of the Th1-promoting genes induced by L. acidophilus NCFM and had an additive effect on genes of the innate immune response and Th2 skewing genes. The gene encoding Jun dimerization protein 2 (JDP2), a transcription factor regulating the activation of JNK, was one of the few genes only induced by B. bifidum Z9. Neutralization of IFN-beta abrogated L. acidophilus NCFM-induced expression of Th1-skewing genes, and blocking of the JNK pathway completely inhibited the expression of IFN-beta. Our results indicate that B. bifidum Z9 actively inhibits the expression of genes related to the adaptive immune system in murine dendritic cells and that JPD2 via blocking of IFN-beta plays a central role in this regulatory mechanism.

General information
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Authors: Fink, L. N. (Intern)
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Influence of the gut microbiota on transcriptional regulation of genes involved in early life development of the intestinal mucus layer

The interplay between the gut microbiota and the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Little is known about how the microbiota regulates mucin proteins, which protect the mucosal surfaces of all epithelial linings by physical hindrance or specific binding of pathogenic agents including virus and bacteria. It has been shown that the presence and composition of the microbiota is directly involved in the regulation of gene transcription in the intestinal epithelium. The intestinal mucus layer of germ free mice has been shown to display a distinctly different composition and structure compared to mucus from conventionally bred animals in vitro and in vivo. This points towards an important role of the microbiota in the regulation of mucin production. To which extent expression of all mucin genes are dependent on the presence of microorganisms and whether specific bacteria are capable of regulating mucus production in early life remains, however, to be established. The very first period after birth is believed to be vulnerable for establishment of the gut microbiota and consequently for the health and integrity of the epithelium throughout life. In this period, a development regulated by endogeneous factors such as hormones, in parallel with gene regulation caused by the microorganisms present in the gut, takes place. Although the microflora undoubtedly plays a regulatory role in the regulation of production of mucin, the importance of endogenous regulation as opposed to gut microbiota has not been investigated. Four groups of mouse pups (n=8 in each group) from differently colonized dams were analyzed with respect to expression of genes involved in mucin production (muc1-4, tff3) in ileal segments isolated on Day 1 and Day 6 after birth. Additionally, the presence of Lactobacillus and E. coli in the ileal samples was assessed by 16S rRNA gene quantification. The pups in the groups were born from dams that were either: 1) germ free (GF), 2) conventional specific pathogen free (SPF), 3) monocolonized with Lactobacillus acidophilus NCFM (Lb NCFM), or 4) monocolonized with E. coli Nissle (E. coli). All data was found by quantitative real-time PCR (qPCR) on Applied Biosystems platforms. Results from these studies showed interesting differences between the four tested animal groups and the two different days tested, which will be presented at the meeting. This is the first study to examine effects of different colonizing bacteria on mucus related gene expression levels in new born mice. These results may thus improve our understanding of the complex interplay between the gut microbiota and epithelial development in the very early life phases.

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Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frækær, H. (Ekstern), Licht, T. R. (Intern)
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Influence of the gut microbiota on transcriptional regulation of genes involved in early life development of the intestinal mucus layer

The interplay between the gut microbiota and the intestinal mucus layer is important both in the maintenance of the epithelial barrier as part of the innate immune defense, and in the conservation of gut homeostasis. Little is known about how the microbiota regulates mucin proteins, which protect the mucosal surfaces of all epithelial linings by physical hindrance or specific binding of pathogenic agents including virus and bacteria. It has been shown that the presence and composition of the microbiota is directly involved in the regulation of gene transcription in the intestinal epithelium. The intestinal mucus layer of germ free mice has been shown to display a distinctly different composition and structure compared to mucus from conventionally bred animals in vitro and in vivo. This points towards an important role of the microbiota in the regulation of mucin production. To which extent expression of all mucin genes are dependent on the presence of microorganisms and whether specific bacteria are capable of regulating mucus production in early life remains, however, to be established. The very first period after birth is believed to be vulnerable for establishment of the gut microbiota and consequently for the health and integrity of the epithelium throughout life. In this period, a development regulated by endogeneous factors such as hormones, in parallel with gene regulation caused by the microorganisms present in the gut, takes place. Although the microflora undoubtedly plays a regulatory role in the regulation of production of mucin, the importance of endogenous regulation as opposed to gut microbiota has not been investigated. Four groups of mouse pups (n=8 in each group) from differently colonized dams were analyzed with respect to expression of genes involved in mucin production (muc1-4, tff3) in ileal segments isolated on Day 1 and Day 6 after birth. Additionally, the presence of Lactobacillus and E. coli in the ileal samples was assessed by 16S rRNA gene quantification. The pups in the groups were born from dams that were either: 1) germ free (GF), 2) conventional specific pathogen free (SPF), 3)
monocolonized with Lactobacillus acidophilus NCFM (Lb NCFM), or 4) monocolonized with E. coli Nissle (E. coli). All data was found by quantitative real-time PCR (qPCR) on Applied Biosystems platforms. Results from these studies showed interesting differences between the four tested animal groups and the two different days tested, which will be presented at the meeting. This is the first study to examine effects of different colonizing bacteria on mucus related gene expression levels in new born mice. These results may thus improve our understanding of the complex interplay between the gut microbiota and epithelial development in the very early life phases.

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Authors: Bergström, A. (Intern), Kristensen, M. B. (Intern), Metzdorff, S. B. (Ekstern), Fink, L. N. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
Publication date: 2010

Lactobacillus acidophilus induces a slow but more sustained chemokine and cytokine response in naïve foetal enterocytes compared to commensal Escherichia coli

The first exposure to microorganisms at mucosal surfaces is critical for immune maturation and gut health. Facultative anaerobic bacteria are the first to colonise the infant gut, and the impact of these bacteria on intestinal epithelial cells (IEC) may be determinant for how the immune system subsequently tolerates gut bacteria. RESULTS: To mirror the influence of the very first bacterial stimuli on infant IEC, we isolated IEC from mouse foetuses at gestational day 19 and from germfree neonates. IEC were stimulated with gut-derived bacteria, Gram-negative Escherichia coli Nissle and Gram-positive Lactobacillus acidophilus NCFM, and expression of genes important for immune regulation was measured together with cytokine production. E. coli Nissle and L. acidophilus NCFM strongly induced chemokines and cytokines, but with different kinetics, and only E. coli Nissle induced down-regulation of Toll-like receptor 4 and up-regulation of Toll-like receptor 2.

The sensitivity to stimulation was similar before and after birth in germ-free IEC, although Toll-like receptor 2 expression was higher before birth than immediately after. CONCLUSIONS: In conclusion, IEC isolated before gut colonisation occurs at birth, are highly responsive to stimulation with gut commensals, with L. acidophilus NCFM inducing a slower, but more sustained response than E. coli Nissle. E. coli may induce intestinal tolerance through very rapid up-regulation of chemokine and cytokine genes and down-regulation of Toll-like receptor 4, while regulating also responsiveness to Gram-positive bacteria.

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Authors: Zeuthen, L. (Intern), Fink, L. N. (Intern), Metzdorff, S. B. (Ekstern), Kristensen, M. B. (Intern), Licht, T. R. (Intern), Nelleman, C. L. (Intern), Frøkiær, H. (Intern)
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Pseudomonas aeruginosa quorum-sensing signal molecules interfere with dendritic cell-induced T-cell proliferation

Pseudomonas aeruginosa releases a wide array of toxins and tissue-degrading enzymes. Production of these malicious virulence factors is controlled by interbacterial communication in a process known as quorum sensing. An increasing body of evidence reveals that the bacterial signal molecule N-(3-oxododecanoyl)-l-homoserine lactone (OdDHL) exhibits both quorum-sensing signalling and immune-modulating properties. Recently, yet another quorum-sensing signal molecule, the Pseudomonas quinolone signal (PQS), has been shown to affect cytokine release by mitogen-stimulated human T cells. In the present article we demonstrate that both OdDHL and PQS decrease the production of interleukin-12 (IL-12) by Escherichia coli lipopolysaccharide-stimulated bone marrow-derived dendritic cells (BM-DCs) without altering their IL-10 release. Moreover, BM-DCs exposed to PQS and OdDHL during antigen stimulation exhibit a decreased ability to induce T-cell proliferation in vitro. Collectively, this suggests that OdDHL and PQS change the maturation pattern of stimulated DCs away from a proinflammatory T-helper type I directing response, thereby decreasing the antibacterial activity of the adaptive immune defence. OdDHL and PQS thus seem to possess dual activities in the infection process: as inducers of virulence factors as well as immune-modulators facilitating the infective properties of this pathogen.
Dendritic Cells from Peyer's Patches and Mesenteric Lymph Nodes Differ from Spleen Dendritic Cells in their Response to Commensal Gut Bacteria

Commensal gut bacteria have potent effects on the immune system, which are partially mediated by intestinal dendritic cells (DC). Distinct commensals confer different properties to in vitro-generated DC. The aim of the present study was to reveal strain-dependent maturation patterns in primary DC. To this end, we compared the response of mouse Peyer's patch (PP) DC, mesenteric lymph node (MLN) DC and spleen DC to the commensal bacteria, Bifidobacterium longum Q46, Lactobacillus acidophilus X37 and Escherichia coli Nissle 1917. Bacterial maturation of DC occurred independently of tissue origin. Expression of CCR7 and CD103 on the surface of MLN DC, necessary for the induction of gut-homing regulatory T cells, increased with stimulation by Gram-positive commensals. Bacteria-dependent cytokine production (IL-6, IL-10 and TNF-alpha) was similar in spleen and MLN DC, and contaminant cells in these DC preparations produced IFN-gamma in response to L. acidophilus. In contrast, PP DC produced IL-6 only in response to E. coli, little IL-10 and no TNF-alpha, and this low cytokine production was not due to inhibition by IL-10 or TGF-beta. Bifidobacteria downregulate IL-6, TNF-alpha and IL-12 production induced in in vitro-generated DC by L. acidophilus. Similar inhibition was observed in splenic DC, but not in MLN DC. MLN cells responded to bacterial stimulation with higher IFN-gamma production than spleen cells, possibly due to the presence of more responsive natural killer cells. Commensal bacteria therefore play specific roles in the gut immune system distinguishable from the effect they would have if recognized by the systemic immune system.

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Main Research Area: Technical/natural sciences

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Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 0.86 SNIP 0.712 CiteScore 2.05
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Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.88 SNIP 0.749 CiteScore 2.16
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Scopus rating (2011): SJR 0.854 SNIP 0.66 CiteScore 2.06
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Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 0.844 SNIP 0.622
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Impact of first bacterial colonizers on immune system development

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Center for Biological Sequence Analysis, Department of Systems Biology, Division of Toxicology and Risk Assessment
Authors: Kristensen, M. B. (Intern), Fink, L. N. (Intern), Zeuthen, L. (Intern), Metzdorff, S. B. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
Publication date: 2008

Impact of first bacterial colonizers on immune system development and homeostasis

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Center for Biological Sequence Analysis, Department of Systems Biology
Authors: Kristensen, M. B. (Intern), Fink, L. N. (Intern), Metzdorff, S. B. (Intern), Frøkiær, H. (Ekstern), Licht, T. R. (Intern)
Publication date: 2008
Impact of first bacterial colonizers on immune system development and homeostasis

Toll-like receptor 2 and nucleotide-binding oligomerization domain-2 play divergent roles in the recognition of gut-derived lactobacilli and bifidobacteria in dendritic cells

Summary: The gut microbiota is vital in the maintenance of homeostasis in the gut immune system. Its diversity and composition play major roles in relation to allergies and inflammatory bowel diseases, and administration of lactic acid bacteria (LAB), such as lactobacilli and bifidobacteria, has positive effects on these pathologies. However, the mechanisms behind the beneficial effects are largely unknown. Here we reveal divergent roles played by Toll-like receptor-2 (TLR2) and nucleotide-binding oligomerization domain-2 (NOD2) in dendritic cell (DC) recognition of LAB. Murine bone-marrow-derived DC lacking NOD2 produce higher levels of interleukin-10 (IL-10) and reduced levels of IL-12 and tumour necrosis factor-[alpha] (TNF-[alpha]) in response to LAB. This indicates that peptidoglycan is partly responsible for the T helper type 1 skewing effect of certain LAB. Dendritic cells that are TLR2/-/- produce less IL-12 and TNF-[alpha] and more IL-10 in response to some strains of lactobacilli, while they produce more IL-12 and less IL-10 in response to bifidobacteria. The same tendency was found in human monocyte-derived DC. We have previously reported that the weak IL-12-inducing and TNF-[alpha]-inducing bifidobacteria inhibit the T helper type 1 skewing effect induced by strong immunostimulatory lactobacilli. Here we show that this immunoinhibitory effect of bifidobacteria is dependent on TLR2 and independent of NOD2. Moreover, independently of the cytokine pattern induced by intact LAB, cell wall fractions of all LAB, as well as synthetic lipoproteins possess immunoinhibitory capacities in both human and murine DC. These novel findings suggest that LAB act as immunoregulators through interaction of lipoprotein with TLR2 and as immunostimulators through interaction of peptidoglycan with NOD2.
Immunomodulatory properties of probiotic bacteria: Effects on dendritic cells and their interactions with NK cells and T cells

Certain lactic acid bacteria (LAB) are part of the commensal intestinal flora and considered beneficial for health, as they compete with pathogens for adhesion sites in the intestine and ferment otherwise indigestible compounds. Another important property of these so-called probiotic bacteria is the ability to modulate the immune response. This thesis describes the immunomodulatory properties of gut-derived bacterial strains on different antigen-presenting cells, and the effector cell responses elicited by bacterially stimulated antigen-presenting cells in natural killer (NK) cells and T cells.
Autologous NK cells and mature dendritic cells (DC) mutually activate each other and this interaction is believed to be important for NK cytotoxic activity against cancer cells and for T cell polarisation. The first study included in this thesis establishes that LAB, as potent stimulators of monocyte-derived DC, are capable of directing NK cell responses. All tested strains increased NK cell proliferation and cytotoxic activity via maturation of DC, whereas only IL-12-inducing LAB induced IFN-gamma production in NK cells. Specific LAB, capable of inhibiting IL-12 production in DC also inhibited IFN-gamma production in NK cells. Secondly, it was investigated whether the strain-dependent induction of IL-12 by LAB and E. coli strains observed in monocyte-derived DC also occurred in freshly isolated blood myeloid DC and monocytes. Both types of blood antigen-presenting cells produced cytokines when stimulated with bacteria, and the cytokine pattern induced by specific bacteria resembled the pattern induced in MoDC, except for TNF-alpha and IL-6, which were induced in response to different bacteria in blood DC/monocytes and monocyte-derived DC. Autologous NK cells produced IFN-gamma when cultured with bacteria, whereas only DC induced IFN-gamma production in allogeneic T cells. In vitro-generated DC is a commonly used model of tissue DC, but they differ in certain aspects from intestinal DC, which are in direct contact with the intestinal microbiota. In the last study, we isolated DC from Peyer’s patches, mesenteric lymph nodes, and spleens of mice, and stimulated these cells with strains of LAB and E. coli. Spleen and mesenteric lymph node DC responded to stimulation with cytokine production comparable to in vitro-generated DC. Peyer’s patch DC produced only IL-6. Cells from spleen and mesenteric lymph nodes enriched in DC rapidly produced IFN-gamma when stimulated with bacteria that induce IFN-gamma production in NK and T cells via in vitro-generated DC. Especially mesenteric lymph node cells produced large amounts of IFN-gamma, which may indicate that mesenteric lymph node NK cells have a strong potential for cytokine-production in response to commensal bacteria.
Distinct gut-derived lactic acid bacteria elicit divergent dendritic cell-mediated NK cell responses

Lactic acid bacteria (LAB) are abundant in the gastrointestinal tract where they continuously regulate the immune system. NK cells are potently activated by dendritic cells (DCs) matured by inflammatory stimuli, and NK cells are present in the gut epithelium and in mesenteric lymph nodes, but it is not known how NK-DC interactions are affected by the predominantly non-pathogenic LAB. We demonstrate that human DCs exposed to different strains of gut-derived LAB consistently induce proliferation, cytotoxicity and activation markers in autologous NK cells. On the contrary, strains of LAB differ greatly in their ability to induce DC-dependent IFN-gamma production by NK cells. This suggests that DCs stimulated by gut LAB may expand the pool of NK cells and increase their cytotoxic potential. Specific LAB, inducing high levels of IL-12 in DCs, may promote amplification of a type-1 response via potent stimulation of IFN-gamma production in NK cells. Combining IFN-gamma-inducing and non-inducing LAB completely abrogates DC-mediated IFN-gamma production by NK cells, and therefore LAB modulating IFN-gamma production in NK cells may be important regulators of the immune response.
Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta

Humans and other mammals coexist with a diverse array of microbes colonizing the intestine, termed the microflora. The relationship is symbiotic, with the microbes benefiting from a stable environment and nutrient supply, and the host gaining competitive exclusion of pathogens and continuously maintenance of the gut immune homeostasis. Here we report novel crosstalk mechanisms between the human enterocyte cell line, Caco2, and underlying human monocyte-derived DC in a transwell model where Gram-positive (G+) commensals prevent Toll-like receptor-4 (TLR4)-dependent Escherichia coli-induced semimaturation in a TLR2-dependent fashion. These findings add to our understanding of the hypo-responsiveness of the gut epithelium towards the microflora. Gut DC posses a more tolerogenic phenotype than conventional DC. Here we show that Caco2 spent medium (SM) induces tolerogenic DC with lower expression of maturation markers, interleukin (IL)-12p70, and tumour necrosis factor-alpha when matured with G+ and Gram-negative (G-) commensals, while IL-10 production is enhanced in DC upon encountering G+ commensals and reduced upon encountering G- bacteria. The Caco2 SM-induced tolerogenic phenotype is also seen in DC priming of naive T cells with elevated levels of transforming growth factor-beta (TGF-beta) and markedly reduced levels of bacteria-induced interferon-gamma production. Caco2 cell production of IL-8, thymic stromal lymphopoietin (TSLP) and TGF-beta increases upon microbial stimulation in a strain dependent manner. TSLP and TGF-beta co-operate in inducing the tolerogenic DC phenotype but other mediators might be involved.
Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through the distinct action of thymic stromal lymphopoietin and transforming growth factor beta
Human antigen-presenting cells respond differently to gut-derived probiotic bacteria but mediate similar strain-dependent NK and T cell activation

The intestinal microbiota is essential for homeostasis of the local and systemic immune system, and particularly strains of lactic acid bacteria and Escherichia coli have been shown to have balancing effects on inflammatory conditions such as allergy and inflammatory bowel disease. However, in vitro assessment of the immunomodulatory effects of distinct strains may depend strongly on the cell type used as a model. To select the most appropriate model for screening of beneficial bacteria in human cells, the response to strains of intestinal bacteria of three types of antigen-presenting cells (APC) was compared; blood myeloid dendritic cells (DC), monocyte-derived DC and monocytes, and the effector response of natural killer cells and naïve T cells was characterized. Maturation induced by gut-derived bacteria differed between APC, with blood DC and monocytes responding with the production of IL-6 and tumour necrosis factor-alpha to bacteria, which elicited mainly IL-10 in monocyte-derived DC. In contrast, comparable IFN-gamma production patterns were found in both natural killer cells and T cells induced by all bacteria-matured APC. An inhibitory effect of certain strains on this IFN-gamma production was also mediated by all types of APC. The most potent responses were induced by monocyte-derived DC, which thus constitute a sensitive screening model.
Probiotic bacteria interact differently with dendritic cells from gut and spleen

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ISI indexed (2011): ISI indexed yes
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BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.756 SNIP 0.84
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.668 SNIP 0.899
Distinct Gut-Derived Bacteria Differentially Affect Three Types of Antigen-Presenting Cells and Impact on NK- and T-Cell Responses

Objectives Gut bacteria are assumed essential for development and maintenance of a balanced immune system. Specifically, stimulation of antigen-presenting cells (APCs) by gut bacteria is important for polarisation of the immune response. This experiment was designed to reveal similarities and differences between the reaction patterns of three types of human APCs when stimulated with intestinal bacteria. Furthermore, the effect of these APCs on NK-cells and T-cells was examined. Methodology The APCs used in this study were blood monocytes, blood dendritic cells, and dendritic cells differentiated from monocytes. Monocyte-derived dendritic cells constitute a commonly used model of dendritic cell function. The APCs were cultured for 18 h with four different gut bacteria: Lactobacillus acidophilus X37, Lactobacillus reuteri DSM 12246, E. coli Nissle 1917 or Bifidobacterium longum Q46. Results & Discussion To examine the polarising effect of gut bacteria on APCs, surface markers and cytokines were measured. The co-stimulatory molecules CD40 and CD86 were induced to a different extent together with CD83. Interleukin-12 (a Th1 cytokine) was only induced by Lactobacillus acidophilus. Interleukin-10, which promotes the development of regulatory T-cells, was mainly induced by the other bacteria. Interleukin-6 and tumour necrosis factor are pro-inflammatory cytokines, often induced by pathogens, but also by some gut bacteria. The effect of the four gut bacteria on monocyte-derived dendritic cells has previously been examined, but this study revealed that their effect on other kinds of APCs is markedly different. When APCs matured by different bacteria were added to either NK-cells or T-cells, different APCs combined with distinct strains of bacteria caused the production of varying amounts of cytokines. Conclusions Distinct gut bacteria possess individual properties leading to different effects on APCs, NK-cells and T-cells. Because NK-cells play a major role in T-cell polarisation, and because the APCs affect T-cells directly, gut bacteria may be very important in maintaining a balanced immune response through these mechanisms. The bacteria examined can potentially be used in tailored probiotic foods exploring their immunomodulatory properties.
Natural Killer Cells are Activated by Lactic Acid Bacteria-Matured Dendritic Cells

Background: Natural killer (NK) cells are lymphocytes of the non-specific immune system recognizing cancerous cells and cells altered by viral infection. Recently, it was proposed that a non-cytolytic subset of NK cells serves a regulatory role by secreting cytokines, possibly affecting both antigen presenting cells and T-cells. Bacteria translocating across the gastrointestinal mucosa are presumed to gain access to NK cell compartments, as consumption of certain strains of lactic acid bacteria has been shown to increase in vivo NK cytotoxic activity. On-going research in our lab aims at describing strain-dependent effects of lactic acid bacteria on regulatory functions of NK-cells. Here, we have investigated how human gut flora-derived non-pathogenic lactic acid bacteria affect NK cells in vitro, by measuring proliferation and IFN-gamma production of human peripheral blood NK cells upon bacterial stimulation.

Methods: CD3-CD56+ NK cells were isolated from buffy coats by negative isolation using a lineage specific antibody cocktail and magnetic beads binding the labelling antibodies on non-NK cells. NK cells were incubated either with 10 microg/ml UV-inactivated lactic acid bacteria or 10 microg/ml phytohemagglutinin (PHA) as a proliferation control. Proliferation was assessed by incorporation of radioactive thymidine into NK cell DNA. Cytokine concentrations were determined by ELISA.

Results: Co-incubation of NK cells and a Lactobacillus acidophilus strain for four days caused increased proliferation of the NK cells and induced IFN-gamma production, both to levels comparable to PHA stimulation. The proliferative response was further enhanced when autologous monocytes were present, probably because cytokines secreted by monocytes having engulfed bacteria stimulated the growth of the NK cells. In contrast, a Lactobacillus paracasei strain caused the NK cells to proliferate only in the presence of monocytes. Conclusion: In this study we have demonstrated that various strains of gut flora-derived lactic acid bacteria have the capacity to activate NK cells in vitro, in a monocyte dependent or independent way. Our results indicate that if NK cells encounter lactic acid bacteria or components hereof in the gut mucosa, this affects NK cell activation by inducing proliferation and cytokine production. Such activation of NK cells may potentially skew an on-going or subsequent immune response towards a Th1 response.
Lactobacilli Differentially Activate Natural Killer Cells

Bacteria translocating across the gastrointestinal mucosa are presumed to gain access to NK cell compartments, as consumption of certain lactic acid bacteria has been shown to increase in vivo NK cytotoxicity. On-going research in our lab aims at describing strain-dependent effects of lactic acid bacteria on regulatory functions of NK-cells. Here, we have investigated how human gut flora-derived non-pathogenic lactobacilli affect NK cells in vitro, by measuring proliferation and IFN-gamma production of human peripheral blood NK cells upon bacterial stimulation. CD3-CD56+ NK cells were isolated from buffy coats by negative isolation using non-NK lineage specific antibodies and magnetic beads. NK cells were incubated with 10 microg/ml UV-inactivated bacteria for four days. Proliferation was assessed by incorporation of radioactive thymidine into NK cell DNA and cytokine concentrations were determined by ELISA. Co-incubation of NK cells and a Lactobacillus acidophilus strain caused increased proliferation of the NK cells and induced IFN-gamma production. The proliferative response was further enhanced in the presence of autologous monocytes, probably because cytokines, secreted by monocytes having engulfed bacteria, stimulated the growth of the NK cells. In contrast, a Lactobacillus paracasei strain caused the NK cells to proliferate only in the presence of monocytes. These results demonstrate that various lactobacilli have the capacity to activate NK cells in vitro, in a monocyte dependent or independent way. Such activation of NK cells may potentially skew an on-going or subsequent immune response towards a Th1 response.

Lactobacilli Modulate Natural Killer Cell Responses In Vitro

Natural killer (NK) cells are cells of the non-specific immune system lysing altered self-cells. A non-cytolytic subset of NK cells may serve a regulatory role by secreting cytokines. Bacteria translocating across the gastrointestinal mucosa are presumed to gain access to NK cells, as consumption of certain lactic acid bacteria has been shown to increase in vivo NK cytotoxicity. Here, we investigated how human gut flora-derived lactobacilli affect NK cells in vitro, by measuring proliferation and IFN-gamma production of human NK cells upon bacterial stimulation. CD3-CD56+ NK cells were isolated from buffy coats by negative isolation using non-NK lineage specific antibodies and magnetic beads. NK cells were incubated with 10 microg/ml UV-inactivated bacteria or 10 microg/ml phytohemagglutinin (PHA) for four days. Proliferation was assessed by incorporation of radioactive thymidine into NK cell DNA. The IFN-gamma concentration was measured by ELISA. Incubation of NK cells with a Lactobacillus acidophilus strain increased the proliferation of the NK cells and induced IFN-gamma production, both to levels comparable to PHA stimulation. The proliferative response was further enhanced with autologous monocytes present, probably because cytokines, secreted by monocytes having engulfed bacteria, stimulated the NK cells. In contrast, a Lactobacillus paracasei strain caused the NK cells to proliferate only in the presence of monocytes. These results demonstrate that various strains of lactobacilli have the capacity to activate NK cells in vitro, in a monocyte dependent or independent way. Hence, the encounter of NK cells with lactic acid bacteria will affect NK cell activation. Such activation of NK cells may potentially skew an on-going or subsequent immune response towards a Th1 response.
Natural Killer Cells Are Activated by Lactic Acid Bacteria-Matured Dendritic Cells

Natural killer (NK) cells are cells of the non-specific immune system lysing altered self-cells. A non-cytolytic subset of NK cells may serve a regulatory role by secreting cytokines. Bacteria translocating across the gastrointestinal mucosa are presumed to gain access to NK cells, as consumption of certain lactic acid bacteria has been shown to increase in vivo NK cytotoxicity. Here, we investigated how human gut flora-derived lactobacilli affect NK cells in vitro, by measuring proliferation and IFN-gamma production of human NK cells upon bacterial stimulation. Human peripheral blood NK cells were incubated with 10 microg/ml UV-inactivated bacteria or 10 microg/ml phytohemagglutinin (PHA) for four days. Proliferation was assessed by incorporation of radioactive thymidine into NK cell DNA. The IFN-gamma concentration was measured by ELISA. Incubation of NK cells with a Lactobacillus acidophilus strain increased the proliferation of the NK cells and induced IFN- production, both to levels comparable to PHA stimulation. The proliferative response was further enhanced with autologous monocytes present, probably because cytokines, secreted by monocytes having engulfed bacteria, stimulated the NK cells. In contrast, a Lactobacillus paracasei strain caused the NK cells to proliferate only in the presence of monocytes. These results demonstrate that various strains of lactobacilli have the capacity to activate NK cells in vitro, in a monocyte dependent or independent way. Hence, the encounter of NK cells with lactic acid bacteria will affect NK cell activation. Such activation of NK cells may potentially skew an on-going or subsequent immune response towards a Th1 response.

General information
State: Published
Organisations: Department of Systems Biology
Authors: Fink, L. N. (Intern), Christensen, H. R. (Intern), Frøkjær, H. (Intern)
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Source-ID: 189394
Publication: Research - peer-review › Poster – Annual report year: 2004

Projects:

Nutritional Immunology
This project runs under the FoodDTU umbrella, and one of its purposes is to create new collaborations between different DTU institutes with ongoing research related to food science. The participating institutes are DTU-Food, DTU-Biosys and DTU-Aqua. The purpose is to elucidate the impact of specific dietary components including e.g. fish oil on the intestinal microbiota and thereby on the development of the immune system in early life. The results are expected to create a basis for better nutritional advice for pregnant women.

National Food Institute
Department of Systems Biology
University of Copenhagen
Number of participants: 14
Project participant:
Kristensen, Matilde Bylov (Intern)
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Nellemann, Christine (Intern)
Kaln, Charlotte (Intern)
Jacobsen, Charlotte (Intern)
Nielsen, Nina Skall (Intern)
Horn, Anna Frisenfeldt (Intern)
Mathiassen, Jakob Hovalt (Intern)
Hellgren, Lars (Intern)
Fink, Lisbeth Nielsen (Intern)
Frøkjær, Hanne (Ekstern)
Broeng Metzdorff, Stine (Ekstern)
Project Manager, organisational:
Licht, Tine Rask (Intern)
Project
Impact of Colonization on immune System Development

National Food Institute
Period: 01/08/2007 → 15/10/2007
Number of participants: 4
Phd Student:
Jensen, Hasse Brønnum (Intern)
Supervisor:
Fink, Lisbeth Nielsen (Intern)
Frøkiær, Hanne (Intern)
Main Supervisor:
Licht, Tine Rask (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Globaliseringsmidler
Project: PhD

Nutritional Immunology
This project runs under the FoodDTU umbrella, and one of its purposes is to create new collaborations between different DTU institutes with ongoing research related to food science. The participating institutes are DTU-Food, DTU-Biosys and DTU-Aqua. The purpose is to elucidate the impact of specific dietary components including e.g. fish oil on the intestinal microbiota and thereby on the development of the immune system in early life. The results are expected to create a basis for better nutritional advice for pregnant women.

National Food Institute
Department of Systems Biology
Period: 01/08/2007 → 31/12/2011
Number of participants: 13
Project participant:
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Bergström, Anders (Intern)
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Kølln, Charlotte (Intern)
Jacobsen, Charlotte (Intern)
Nielsen, Nina Skall (Intern)
Horn, Anna Frisenfeldt (Intern)
Mathiassen, Jakob Hovalt (Intern)
Hellgren, Lars (Intern)
Fink, Lisbeth Nielsen (Intern)
Project Manager, organisational:
Licht, Tine Rask (Intern)

Nutritional Immunology
National Food Institute
Department of Systems Biology
National Institute of Aquatic Resources
Period: 04/01/2007 → 31/12/2011
Number of participants: 10
Project participant:
Wilcks, Andrea (Intern)
Bergström, Anders (Intern)
Andersen, Jens Bo (Intern)
Metzdorff, Stine Broeng (Intern)
Fink, Lisbeth Nielsen (Intern)
Nielsen, Nina Skall (Intern)
Project Manager, organisational:
Licht, Tine Rask (Intern)
Frøkiær, Hanne (Intern)
Hellgren, Lars (Intern)
Jacobsen, Charlotte (Intern)

Financing sources
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Name of research programme: [Ordinær drift UK 10]
Amount: 3,250,000.00 Danish Kroner

Effects of bacterial colonization on immune maturation
The Gut Ecology group at the National Food Institute, Technical University of Denmark investigates effects of bacterial colonization on the maturation of the immune system in early life.

We do this by use of germ-free and monoclonized mouse models.

The project is closely related to other projects in the Gut Ecology research group, where we analyze the intestinal microbiota in infants.

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Globalization funds (through FoodDTU)
National Food Institute
Division of Food Microbiology
Communications and Management Secretariat
Period: 01/01/2007 → 01/01/2012
Number of participants: 7
Number of related Ph.D. students: 1
Project participant:
Bergström, Anders (Intern)
Nellemann, Christine (Intern)
Frøkiær, Hanne (Intern)
Metzdorff, Stine Broeng (Intern)
Fink, Lisbeth Nielsen (Intern)
Project Manager, organisational:
Licht, Tine Rask (Intern)
Phd Student:
Kristensen, Matilde Bylov (Intern)

Immunomodulating Properties of Probiotic Bacteria
Department of Systems Biology
Number of participants: 6
Phd Student:
Fink, Lisbeth Nielsen (Intern)
Supervisor:
Christensen, Hanne Risager (Intern)
Main Supervisor:
Frøkiær, Hanne (Intern)
Examiner:
Lund, Ole (Intern)
De Jong, Esther C. (Ekstern)
Hokland, Marianne (Ekstern)

**Financing sources**

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Project: PhD