
English Summary

The mucosal immune system has the responsible task to maintain mucosal homeostasis, allowing tolerance against harmless antigens, derived from diet or commensal bacteria, but effective against pathogens and pathogenic antigens. Several environmental factors, such as dietary metabolites and microbial derived products, which are present in the intestinal lumen and thus in close proximity with the mucosal immune system, have been indicated as factors that can influence this homeostasis. Moreover, the host is able to regulate the composition of the microbiota present in the intestines with the production of e.g. defensins and mucus¹⁻³, while the microbiota influences the available nutrition for the host by diet fermentation^{4,5}. On top of all this, the nutritional intake of an individual also affects the composition of the microbiota⁶⁻⁸. Therefore, the regulation of the mucosal immune system is complex and can either be directly or indirectly modulated by various environmental factors.

The effect of vitamin A on the mucosal immune system.

A crucial role for vitamin A in the regulation of various processes that mediate the mucosal homeostasis are well documented. For instance, the tolerogenic phenotype of dendritic cells (DCs) within the mucosal immune system is dependent on the formation of the active metabolite of vitamin A, retinoic acid (RA), produced by intestinal epithelial cells⁹⁻¹¹. Consequently, these DCs can in turn produce high amounts of RA, which induces regulatory T-cells, IgA producing B-cells and gut homing molecules, allowing the lymphocytes to migrate to the lamina propria of the small intestines¹²⁻¹⁴. Together, these RA dependent processes control the tolerogenic functions of the mucosal immune system.

In this thesis we demonstrated additional aspects of the mucosal immune system that are regulated by dietary vitamin A. We show in chapter 2 that besides DCs also the differentiation of intestinal macrophages is affected by vitamin A. Although it has been reported that the production of pro-inflammatory cytokines in macrophages and DCs can be blocked by RA¹⁵⁻¹⁷, we established that the expression of Dectin-1 on intestinal macrophages was also dependent on RA. With the expression of the Dectin-1 receptor macrophages exhibited the plasticity to rapidly switch their phenotype upon ligation, which is needed during epithelial disruption and inflammation. Although contradictory results were obtained in Dectin-1 deficient mice, which received DSS induced colitis^{18,19}, links have been made with genetic alterations in the Dectin-1 gene in inflammatory bowel disease (IBD) patients^{19,20}. Dectin-1 is a C-type lectin receptor that upon binding will induce the production of pro-inflammatory cytokines. Whereas Dectin-1 has been shown to recognize bacterial antigens, its receptor is mainly known for its binding to fungi^{21,22}. Because the sequencing techniques, which are used for the analysis of the microflora composition present in the feces of the intestines, are not properly designed for fungi analysis,

studies so far may have missed the role of fungi. In addition, fungi are much bigger in mass compared to bacteria, therefore, although the numbers of fungi are much smaller, they might still have a significant contribution to the homeostasis of the host ²³. In conclusion, this indicates that regulation of the mucosal immune system via fungal signaling should not be underestimated.

Furthermore, a critical role for vitamin A in the formation of secondary lymphoid organs during embryogenesis was shown by our group. Lymphoid organogenesis depends on the clustering of lymphoid tissue inducer (LTi) cells, which belong to the group 3 innate lymphoid cells (ILCs). RA induced the expression of CXCL13 by stromal organizer cells, which recruited LTi cells for the initiation of cluster formation ²⁴. Subsequently the levels of maternal vitamin A further influenced the maturation of ILC progenitors into mature LTi cells within the embryo, determining the size of secondary lymphoid organs that are formed ²⁵. In chapter 3 we have demonstrated that intestinal group 3 ILCs, containing the LTi cells, are reduced upon vitamin A deficiency in adult mice. Our data confirmed recently published results, showing reduced numbers of group 3 ILCs in the intestines when vitamin A was absent ^{26,27}. In addition, it is previously reported that the composition of the microbiota is changed in mice that were vitamin A deficient. These changes in the microbiota, especially the reduction in segmented filamentous bacteria, were shown to suppress the generation of Th17 cells ²⁸. Therefore, we should not exclude the role of the changed composition of the microbiota within these vitamin A deficient mice on the ILC population within the lamina propria. However, we were able to demonstrate that group 3 ILCs were directly affected by RA, as mice in which RA signaling was specifically blocked in ROR γ^+ cells, showed reduced numbers of group 3 ILCs similar to vitamin A deficient mice. Moreover, vitamin A deficient mice postnatally developed fewer intestinal lymphoid structures compared to their control counterparts. Our observations illustrate that vitamin A is not only essential for the development of lymphoid tissue during embryogenesis, but also postnatally for the formation of lymphoid clusters within the intestines of adult mice.

Since group 3 ILCs are the main producers of intestinal IL-22, we and others observed diminished expression of IL-22 in the absence of vitamin A as a consequence of the reduced group 3 ILCs ^{26,27}, (chapter 3). These intestinal IL-22 producing ILCs play a crucial role in host defense against *Citrobacter rodentium* infections ²⁹. As a result it has been demonstrated that vitamin A deficient mice were more susceptible to *Citrobacter rodentium* infection, while RA was able to reduce the severity of the infection ^{26,27}. Furthermore, in chapter 4 we demonstrated that BALB/c mice, which are better protected to chemical induced colitis, have a higher vitamin A metabolism compared to C57Bl/6 mice. BALB/c mice showed a higher activity of vitamin A converting enzymes in DCs of the MLN. Consequently the percentages of regulatory T cells present in the intestines and the production of luminal IgA by B cells was increased in BALB/c mice compared to C57Bl/6 mice. Although our data indicate that the difference in vitamin A metabolism between the two different

mouse strains lead to a better protective barrier in BALB/c mice against chemical induced colitis, these mice are also reported to have a different microbiota present in the gut^{30,31}, which also might influence the disease severity within the mice. Additionally we showed that vitamin A deficient mice were less capable of coping with DSS induced colitis. These data are in line with earlier obtained results, demonstrating a crucial role for vitamin A signaling in maintaining the gut homeostasis^{26,27,32-34}.

The influence of the microbiota on the mucosal immune system.

Microbiota, which are consistently present in the lumen of the intestines are able to digest the diet and produce metabolites suitable for the host in which they live in symbiosis^{4,35,36}. While the influence of the microbiota on the immune system of the host is becoming more evident, we demonstrated in chapter 5 that diet derived microbial compounds can modulate the mucosal immune system. Dietary adjustments in the form of fibers showed an increased expression of vitamin A metabolizing enzymes in intestinal epithelial cells, resulting in induced tolerogenic capacity of MLN-DCs. Moreover, we provided evidence that short chain fatty acids (SCFAs), which are fiber derived metabolites, are able to induce RALDH-1 expression in an intestinal epithelial cell line. These SCFAs most likely signal via the inhibition of HDAC, although this should be further addressed with the use of specific HDAC knockout mice.

The role of SCFAs and HDAC inhibition in modulating the mucosal and systemic immune system have been extensively studied in the last decade. SCFAs and HDAC inhibition have been shown to modulate the mucosal immune system with the induction of regulatory T cells, epithelial integrity and additionally may be involved in the secretion of IgA by B-cells³⁷⁻⁴¹. However, these studies do not address whether there is a role of the epithelial cells in modulating the immune system, even though they are the first to be in contact with environmental factors present in the lumen. In addition, *Allenghat et al.* presented a specific role of HDAC 3 in intestinal epithelial cells for maintaining mucosal homeostasis, and dampening intestinal inflammation⁴². Together with our data this illustrates a crucial role for environmental factors to influence epithelial cells, which in turn will modulate the mucosal immune system. SCFA mediated signaling can also occur via the binding to G protein coupled receptors (GPCRs). The lack of GPR43 expression in mice resulted in more severe DSS induced colitis with enhanced numbers of neutrophils. In addition, inflammation during arthritis and asthma were also affected⁴³. These data indicate that SCFAs can affect the mucosal immune system via different pathways, i.e. either via GPCR signaling or through the inhibition of HDAC.

Furthermore, dietary alterations induced differences in the distribution of the microbiota present within the feces of the small intestines. We showed that the presence of specific species of bacteria differed when mice received either a conventional or synthetic diet. Our data, presented in chapter 5, are in line with earlier studies, which compared dietary influences or disease related alterations in the

microbiota. For instance, mice that received either a high fat diet or a low fiber diet showed an enhanced expression of *Erysipelotrichia* bacteria^{44,45}, similar to our mice which were fed a synthetic diet. Patients with inflammatory bowel disease (IBD) often demonstrate alterations in the composition of the microbiota, which correlates with an unhealthy dysbiosis. Therefore, further research on therapeutic innovations, which restores the microbiota distribution in these patients, is very important.

Besides the role of microbiota in the fermentation and production of diet derived products, the mere presence of microbiota can also affect the immune system of the host. Several studies have shown that different cells depend on the presence of bacteria, as germfree or antibiotic treated mice showed alterations in their immune status. For example, the production of IL-1 β by intestinal macrophages, which is critical for Th17 cell development, is dependent on the presence of microbiota^{46,47}. However, the impact of commensal bacteria on ILC development is not completely understood. Although group 1 and group 2 ILCs seem to develop normally in the absence of bacteria^{48,49}, contradictory results have been published concerning the development of ROR γ^+ group 3 ILCs. In the absence of microbiota the development of LTi cells and the formation of secondary lymphoid organs occurs normally within the embryo^{50,51}. Data from adult mice however showed in one study that the presence of microbiota is needed for the production of IL-22 by group 3 ILCs, whereas another study demonstrated that microbiota repressed the production of IL-22 by group 3 ILCs^{52,53}. This indicates that further research is still needed on this topic.

Some species of bacteria have been demonstrated to influence the differentiation of specific cells of the mucosal immune system. Segmented filamentous bacteria were shown to be essential for the induction of Th17 cells. This explained the observation that mice coming from distinct sources have varying numbers of Th17 cells, which correlated with the presence or absence of these bacteria⁵⁴. Moreover, recent studies showed that the specific combinations of several bacteria were able to induce the presence of regulatory T cells, leading to the protection against DSS induced colitis^{55,56}.

It is becoming clear that there is an intricate interaction between microbiota and the mucosal immune system. Therefore, research is now focused on both topics, which led to rapidly increasing insights in the interactions that occur between the microbiota and the host. Nevertheless, additionally research to study the underlying mechanisms that are involved in the generation of mucosal homeostasis is still needed.

Inflammatory bowel disease.

Patients with inflammatory bowel disease (IBD) can be divided into two main groups; patients with Ulcerative Colitis (UC) and patients with Crohn's disease. Both patients suffer from chronic inflammation in the intestinal tract. While UC is mainly located in the colon, Crohn's disease can be found throughout the entire digestive

tract. In both patient groups several studies identified single nucleotide polymorphisms which are associated with an increased risk to develop IBD^{57,58}. In addition, some correlations have been made between IBD patients and their vitamin A metabolism. For instance, a possible relation is illustrated between a polymorphism in the *Cyp26B1* gene, which codes for an enzyme involved in the breakdown of excessive RA and is a direct target gene of RA signaling, inducing a negative feedback loop. Although, this polymorphism could lead to higher concentrations of RA, this potential link was only seen in Crohn's disease patients and not in UC patients⁵⁹. Moreover, studies that investigated the capacity to produce RA by both intestinal DC's and macrophages showed an induced enzymatic activity to metabolize vitamin A in Crohn's patients. Nonetheless, macrophage precursors, present in the blood, showed a reduced enzymatic activity to metabolize vitamin A in Crohn's patients compared to healthy controls⁶⁰. These data indicate that a balanced production of RA is beneficial to induce tolerogenic mechanisms, although excessive production of RA might induce inflammation and can be harmful to the host.

Furthermore, examining the cytokine production of ILCs in Crohn's patients demonstrated an imbalanced ratio of IL-22 and IFN γ producing ILCs, whereas UC patients did not show any differences compared to controls. Crohn's disease was associated with induced IFN γ production, which was mediated via IL-23 production by intestinal macrophages⁶¹. Paradoxical results were published, demonstrating higher levels of IL-22 in Crohn's patients, while IFN γ levels were not changed compared to healthy controls⁶². However, while enhanced IL-22 levels were measured in the blood of Crohn's patients, enhanced IFN γ production by ILCs was measured within the intestines. As vitamin A is indicated to influence the differentiation of ILCs and their cytokine production, these data indicate a role for vitamin A in balancing the intestinal ILC subset in Crohn's patients.

In conclusion, although clear results for the protective mechanism induced by vitamin A metabolism or RA in colitis were shown in mouse models²⁶⁻²⁸, data from patients show that the role of vitamin A metabolism in inflammatory bowel disease is not clear yet and therefore further research is still needed to address these questions.

Colorectal cancer.

Colorectal cancer is one of the most common forms of cancer. Men have a higher risk to develop colorectal cancer compared to females, which increases with age. Other risk factors involved in the development of colorectal cancer are smoking, obesity, family history, physical activity and IBD. Moreover, it is already known for a long time that developing countries have a lower incidence of colorectal cancer compared to western countries⁶³⁻⁶⁵. Meta-analysis showed that specifically dose dependent fiber intake is inversely associated with the development of colorectal adenoma. Colorectal adenoma risk was reduced by 28% when high fiber intake was analyzed⁶⁴. The mechanism of dietary fiber intake that has been proposed to medi-

ate protection against colorectal cancer is the effect of fiber derived SCFAs. SCFAs, produced by the microflora, induce hyper activation of the WNT/ β -catenin pathway and consequently promote cell apoptosis in colorectal cancer cells⁶⁶⁻⁶⁸. Native Africans have higher levels of SCFAs compared to American Africans. Therefore, native Africans from developing countries might have a lower risk to develop colorectal cancer compared to people that live in western countries⁶⁹. Although clinical trials until now have failed to demonstrate that high-fiber consumption protects against colorectal cancer, a protective effect may have been missed, as these trials were only performed in high-risk populations⁶⁴. Clinical trials in cancer patients are now focusing on the mechanisms which are induced by dietary fiber^{70,71}. In addition, high fiber intake can also have beneficial effects in colorectal cancer by influencing the composition of the microbiota and promoting fecal bulking and viscosity, thereby reducing the uptake of carcinogens by intestinal cells⁷².

In addition, other dietary compounds have been recognized to elevate the risk of colorectal cancer development. For instance, the intake of processed meat was shown to increase the risk of colorectal cancer⁶⁵. In addition, obesity, which can be caused by a high fat diet, is associated with a higher risk to develop colorectal cancer. Recently it has been demonstrated that a high fat diet, independently of obesity, enhanced the risk for colorectal cancer. The high fat diet altered the composition of the microbiota, which affected the mucosal immune system and showed an impaired response to tumor growth. Moreover, the disease could be transmitted upon microbiota transfer to healthy transgenic mice, which received a normal diet. With the administration of butyrate the microbiota composition was restored and tumor growth was reduced⁷³. Thus, the fermentation of dietary products executed by the microbiota is not only important for controlling colorectal cancer, it also affects the composition of the microbiota.

As we have demonstrated that changes in diet and microbiota composition affected the vitamin A metabolism in the intestine (chapter 5) and in light of the important association of microbiota and colorectal cancer, vitamin A metabolism might play a crucial role in the development of colorectal cancer. Material of patients with colorectal cancer demonstrated a higher expression of Cyp26a1, Cyp26B1 and LRAT compared to healthy controls. These enzymes are all involved in de degradation of either retinol or RA. A high expression of Cyp26B1 and LRAT were even associated with poor prognosis in colorectal patients⁷⁴. Although they concluded within this article that induced levels of vitamin A or RA degrading enzymes would lead to lower levels of RA, these data also might illustrate that patients have higher levels of RA as these enzymes are induced by RA signaling itself. Nevertheless, an association for vitamin A metabolism and colorectal cancer is indicated.

Environmental factors in other diseases.

Type 2 diabetes is a metabolic disorder that is caused by insulin resistance of the body, leading to high sugar levels in blood. This disorder is associated with both ge-

netic and environmental factors. Recently, with the use of metagenome wide association studies, two research groups were able to identify specific microbial changes in type 2 diabetic patients. Although some differences were found, which could be related to the distinct background of the patients studied, being either from European or Chinese origin, both studies showed specific reduced presence of butyrate producing bacteria. These observations demonstrated a predictive potential of identifying type 2 diabetic patients by the intestinal microbiome^{75,76}. Moreover, one study was able to discriminate between pre-type 2 diabetes and normal glucose tolerance with metagenomic wide association research⁷⁵. The data illustrated a critical role for the microbiota in the development of type 2 diabetes and suggest that patients can be treated by restoring their dysbiotic microbiota.

Other metabolic disorders, which are associated with an altered intestinal microbiota, are obesity and cardiovascular diseases⁵. When the mucosal barrier is defective an endotoxaemia may be induced which will further promote the development of metabolic disorders. Therefore, the production of IL-22 by intestinal ILCs and T cells is essential to maintain the mucosal barrier, as it modulates epithelial barrier functions. It has been demonstrated that mice which have a defect in IL-22 signaling, have indeed higher chances to develop metabolic disorders when they receive a high fat diet. In addition, treatment with IL-22 has been shown to overcome insulin resistance⁷⁷, illustrating that therapeutic intervention aimed at regulating the IL-22 pathway might be beneficial for patients with metabolic disorders. Vitamin A metabolites might thus be very interesting as they enhance IL-22 production^{26,27} (chapter 3).

Intake of dietary fibers has not only been shown to benefit intestinal disorders, as also many other diseases were shown to be dampened by it. Systemic higher levels of SCFAs, which are produced by the microbiota from dietary fibers, showed reduced inflammation in allergic lung diseases. When these findings were further analyzed an enhanced hematopoiesis of DC precursors in the bone marrow was demonstrated, with less activation in the lung upon allergic antigen encounter⁴⁵. These results showed a significant role for systemic levels of SCFAs induced by fiber intake in allergic lung diseases.

Furthermore, obesity, diabetes and cardiovascular diseases are shown to have beneficial effects from a high fiber intake. First of all, dietary fibers are related to a better body mass index by reducing weight. Secondly, fibers regulate the microbiota composition, which often is disbalanced in these patients. And moreover, dietary fiber intake is associated with anti-inflammatory effects⁷⁸. In addition, it has even demonstrated that dietary fiber intake and microbiota composition influences behavior and cognition in both human and mice^{78,79}.

In sum, not only intestinal disorders are influenced by dietary intake and microbiota composition, but also many other conditions and diseases.

Therapeutic implications.

Treatment of microbiota dysbiosis in patients with intestinal disorders reduces clinical symptoms. Additionally, microbiota treatment might even help the systemic immune system in other diseases as type 2 diabetes. While antibiotics were first associated with benefits in IBD patients, it has been demonstrated that antibiotic exposure further enhances the microbiota dysbiosis in IBD patients. Moreover, antibiotic resistance is a growing health concern and therefore antibiotic treatment in a chronic disease is not preferred⁸⁰. With the increased interest in the field of microbiota and their role in regulating the mucosal immune system new insights are revealed. Data are now emerging which specific bacteria or their products might have beneficial effects for the host. Therefore, prebiotics and probiotics are giving new possible therapeutic approaches. While prebiotics stimulate the growth of specific bacteria, probiotics are live bacteria, which both, after intake, should induce health benefits to the host. Not only the live bacteria might have beneficial effects, also bacterial components, dead bacteria and the substances secreted by bacteria have demonstrated health benefits for the host^{81,82}. However, clinical trials showed moderate effects of probiotic treatments, which might be because these treatments are not standardized yet. The optimal dosing and efficacy of certain prebiotics and probiotics should be further investigated in large scale clinical trials^{83,84}. Furthermore, single strain probiotics might have limited effects, while combinations of a few bacteria have a better potential to sustain within the microflora of the host. Several studies demonstrated promising results with combinations of a few bacteria, which were able to induce regulatory T cells and protection against DSS induced colitis in mice with a dysbiotic microbiota^{55,56}. A more resolute treatment would be fecal transplantation. After the right donor is selected, which most of the time will be within the direct surroundings of the patient, the fecal sample will be screened for unknown pathogens. Although this method has shown good results in infection with *Clostridium difficile*, fecal transplantation is an intense treatment for the patient as first the total intestines will be flushed followed by fecal transplantation^{56,85}. Besides therapeutics that directly modulate the microbiota distribution, dietary intake also effects this distribution. Moreover, the diet can both directly and indirectly, after microbiota fermentation, affect the mucosal immune system. Whereas it might be one of the oldest concepts in medicine to promote health via the diet, only within the last decade we now start to understand partly how these mechanisms work. Intake of high fibers are associated with a reduced risk to develop intestinal disorders, but has also been shown to have protective effects against many other diseases. While the recommended fiber intake is 25-28 gram a day, the average American only consumes around 16 gram of fibers a day⁸⁶. This already indicates that the average fiber intake is often too low. Additionally, nutrition in the form of vitamins and Ahr ligands are shown to be beneficial for the host by modulating

the mucosal immune system. Therefore, a healthy varied diet with a high fiber and vitamin intake would stimulate a symbiotic microbiota and modulate the mucosal immune system to be tolerant against harmless antigens^{78,87}.

Concluding remarks.

Overall, within this thesis we have described several processes which modulate the mucosal immune system. We have demonstrated additional affects, which are regulated by vitamin A metabolism, that are of influence for the functioning of the mucosal immune system. Moreover, we presented data that showed both genetic factors and dietary fibers were able to regulate the vitamin A metabolism in the intestines. These results provide new therapeutic strategies to restore and maintain a balanced mucosal immune system by adapting dietary intake and modulate the microbiota. This might not only be helpful for patients with intestinal disorders but also various other inflammatory and metabolic diseases.

Reference List

- 1 Ostaff MJ, Stange EF, Wehkamp J. Antimicrobial peptides and gut microbiota in homeostasis and pathology. *Embo Molecular Medicine* 2013; 5: 1465-83.
- 2 Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: A hypothesis on intimate interplay at the intestinal mucosa. *Seminars in Immunology* 2007; 19: 70-83.
- 3 Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu XF, Koren O et al. The Antibacterial Lectin RegIII γ Promotes the Spatial Segregation of Microbiota and Host in the Intestine. *Science* 2011; 334: 255-8.
- 4 Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism and the immune system. *Nature Immunology* 2013; 14: 676-84.
- 5 Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; 489: 242-9.
- 6 David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; 505: 559-+.
- 7 De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America* 2010; 107: 14691-6.
- 8 Zoetendal EG, de Vos WM. Effect of diet on the intestinal microbiota and its activity. *Current Opinion in Gastroenterology* 2014; 30: 189-95.
- 9 Edele F, Molenaar R, Guetle D, Dudda JC, Jakob T, Homey B et al. Instructive role of peripheral tissue cells in the imprinting of T cell homing receptor patterns. *Journal of Immunology* 2008; 181: 3745-9.
- 10 Iliev ID, Mileti E, Matteoli G, Chieppa M, Rescigno M. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal Immunology* 2009; 2: 340-50.
- 11 McDonald KG, Leach MR, Brooke KWM, Wang CH, Wheeler LW, Hanly EK et al. Epithelial Expression of the Cytosolic Retinoid Chaperone Cellular Retinol Binding Protein II Is Essential for in Vivo Imprinting of Local Gut Dendritic Cells by Luminal Retinoids. *American Journal of Pathology* 2012; 180: 984-97.
- 12 Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004; 21: 527-38.
- 13 Molenaar R, Knippenberg M, Goverse G, Olivier BJ, de Vos AF, O'Toole T et al. Expression of Retinaldehyde Dehydrogenase Enzymes in Mucosal Dendritic Cells and Gut-Draining Lymph Node Stromal Cells Is Controlled by Dietary Vitamin A. *Journal of Immunology* 2011; 186: 1934-42.
- 14 Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006; 314: 1157-60.
- 15 Na SY, Kang BY, Chung SW, Han SJ, Ma XJ, Trinchieri G et al. Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NF kappa B. *Journal of Biological Chemistry* 1999; 274: 7674-80.
- 16 Wada Y, Hisamatsu T, Kamada N, Okamoto S, Hibi T. Retinoic Acid Contributes to the Induction of IL-12-Hypoproducing Dendritic Cells. *Inflammatory Bowel Diseases* 2009; 15: 1548-56.
- 17 Wang XC, Allen C, Ballow M. Retinoic acid enhances the production of IL-10 while reducing the synthesis of IL-12 and TNF-alpha from LPS-stimulated monocytes/macrophages. *Journal of Clinical Immunology* 2007; 27: 193-200.
- 18 Heinsbroek SEM, Oei A, Roelofs JJTH, Dhawan S, te Velde A, Gordon S et al. Genetic deletion of dectin-1 does not affect the course of murine experimental colitis. *Bmc Gastroenterology* 2012; 12.
- 19 Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP et al. Interactions Between

-
- Commensal Fungi and the C-Type Lectin Receptor Dectin-1 Influence Colitis. *Science* 2012; 336: 1314-7.
- 20 Cunha C, Di Ianni M, Bozza S, Giovannini G, Zagarella S, Zelante T et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* 2010; 116: 5394-402.
 - 21 Chamilos G, Ganguly D, Lande R, Gregorio J, Meller S, Goldman WE et al. Generation of IL-23 Producing Dendritic Cells (DCs) by Airborne Fungi Regulates Fungal Pathogenicity via the Induction of T-H-17 Responses. *Plos One* 2010; 5.
 - 22 Ferwerda G, Meyer-Wentrup F, Kullberg BJ, Netea MG, Adema GJ. Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages. *Cellular Microbiology* 2008; 10: 2058-66.
 - 23 Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. *Nat Rev Immunol* 2014; 14: 405-16.
 - 24 van de Pavert SA, Olivier BJ, Goverse G, Vondenhoff MF, Greuter M, Beke P et al. Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nature Immunology* 2009; 10: 1193-U78.
 - 25 van de Pavert SA, Ferreira M, Domingues RG, Ribeiro H, Molenaar R, Moreira-Santos L et al. Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. *Nature* 2014; 508: 123-+.
 - 26 Mielke LA, Jones SA, Raverdeau M, Higgs R, Stefanska A, Groom JR et al. Retinoic acid expression associates with enhanced IL-22 production by gamma delta T cells and innate lymphoid cells and attenuation of intestinal inflammation. *Journal of Experimental Medicine* 2013; 210: 1117-24.
 - 27 Spencer SP, Wilhelm C, Yang Q, Hall JA, Bouladoux N, Boyd A et al. Adaptation of Innate Lymphoid Cells to a Micronutrient Deficiency Promotes Type 2 Barrier Immunity. *Science* 2014; 343: 432-7.
 - 28 Cha HR, Chang SY, Chang JH, Kim JO, Yang JY, Kim CH et al. Downregulation of Th17 Cells in the Small Intestine by Disruption of Gut Flora in the Absence of Retinoic Acid. *Journal of Immunology* 2010; 184: 6799-806.
 - 29 Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nature Medicine* 2008; 14: 282-9.
 - 30 Hildebrand F, Nguyen TLA, Brinkman B, Yunta RG, Cauwe B, Vandenaabeele P et al. Inflammation-associated enterotypes, host genotype, cage and inter-individual effects drive gut microbiota variation in common laboratory mice. *Genome Biology* 2013; 14.
 - 31 Jussi V, Erkki E, Paavo T. Comparison of cellular fatty acid profiles of the microbiota in different gut regions of BALB/c and C57BL/6J mice. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 2005; 88: 67-74.
 - 32 Bai AP, Lu NH, Guo Y, Liu ZJ, Chen J, Peng ZK. All-trans retinoic acid down-regulates inflammatory responses by shifting the Treg/Th17 profile in human ulcerative and murine colitis. *Journal of Leukocyte Biology* 2009; 86: 959-69.
 - 33 Gangopadhyay IH, Moldoveanu Z, Stephensen CB. Vitamin A deficiency has different effects on immunoglobulin A production and transport during influenza A infection in BALB/c mice. *Journal of Nutrition* 1996; 126: 2960-7.
 - 34 Hong K, Zhang Y, Guo Y, Xie J, Wang J, He X et al. All-trans retinoic acid attenuates experimental colitis through inhibition of NF- κ B signaling. *Immunology Letters*.
 - 35 Schluter J, Foster KR. The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection. *Plos Biology* 2012; 10.
 - 36 Spencer SP, Belkaid Y. Dietary and commensal derived nutrients: shaping mucosal and systemic immunity. *Current Opinion in Immunology* 2012; 24: 379-84.
 - 37 Arpaia N, Campbell C, Fan XY, Dikiy S, van der Veeken J, deRoos P et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; 504: 451-+.
 - 38 de Zoeten EF, Wang LQ, Sai H, Dillmann WH, Hancock WW. Inhibition of HDAC9 Increases T

-
- Regulatory Cell Function and Prevents Colitis in Mice. *Gastroenterology* 2010; 138: 583-94.
- 39 de Zoeten EF, Wang LQ, Butler K, Beier UH, Akimova T, Sai H et al. Histone Deacetylase 6 and Heat Shock Protein 90 Control the Functions of Foxp3(+) T-Regulatory Cells. *Molecular and Cellular Biology* 2011; 31: 2066-78.
- 40 Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; 504: 446-+.
- 41 Ishikawa T, Nanjo F. Dietary Cycloinulooligosaccharides Enhance Intestinal Immunoglobulin A Production in Mice. *Bioscience Biotechnology and Biochemistry* 2009; 73: 677-82.
- 42 Alenghat T, Osborne LC, Saenz SA, Kobuley D, Ziegler CGK, Mullican SE et al. Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. *Nature* 2013; 504: 153-+.
- 43 Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; 461: 1282-U119.
- 44 Greiner T, Backhed F. Effects of the gut microbiota on obesity and glucose homeostasis. *Trends in Endocrinology and Metabolism* 2011; 22: 117-23.
- 45 Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine* 2014; 20: 159-66.
- 46 Franchi L, Kamada N, Nakamura Y, Burberry A, Kuffa P, Suzuki S et al. NLR4-driven production of IL-1 beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. *Nature Immunology* 2012; 13: 449-U45.
- 47 Shaw MH, Kamada N, Kim YG, Nunez G. Microbiota-induced IL-1 beta, but not IL-6, is critical for the development of steady-state T(H)17 cells in the intestine. *Journal of Experimental Medicine* 2012; 209: 251-8.
- 48 Ganal SC, Sanos SL, Kalfass C, Oberle K, Johner C, Kirschning C et al. Priming of Natural Killer Cells by Nonmucosal Mononuclear Phagocytes Requires Instructive Signals from Commensal Microbiota. *Immunity* 2012; 37: 171-86.
- 49 Monticelli LA, Sonnenberg GF, Artis D. Innate lymphoid cells: critical regulators of allergic inflammation and tissue repair in the lung. *Current Opinion in Immunology* 2012; 24: 284-9.
- 50 Mebius RE, Rennert P, Weissman IL. Developing lymph nodes collect CD4(+)CD3(-) LT beta(+) cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity* 1997; 7: 493-504.
- 51 van de Pavert SA, Mebius RE. New insights into the development of lymphoid tissues. *Nature Reviews Immunology* 2010; 10: 664-U24.
- 52 Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C et al. ROR gamma t and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46(+) cells. *Nature Immunology* 2009; 10: 83-91.
- 53 Sawa S, Lochner M, Satoh-Takayama N, Dulauroy S, Berard M, Kleinschek M et al. ROR gamma t(+) innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nature Immunology* 2011; 12: 320-U71.
- 54 Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* 2009; 139: 485-98.
- 55 Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H et al. T-reg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; 500: 232-+.
- 56 Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C et al. Targeted Restoration of the Intestinal Microbiota with a Simple, Defined Bacteriotherapy Resolves Relapsing Clostridium difficile Disease in Mice. *Plos Pathogens* 2012; 8.
- 57 Beaudoin M, Goyette P, Boucher G, Lo KS, Rivas MA, Stevens C et al. Deep Resequencing of GWAS Loci Identifies Rare Variants in CARD9, IL23R and RNF186 That Are Associated with Ulcerative Colitis. *Plos Genetics* 2013; 9.
- 58 Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; 448: 427-34.

-
- 59 Fransen K, Franzen P, Magnuson A, Elmabsout AA, Nyhlin N, Wickbom A et al. Polymorphism in the Retinoic Acid Metabolizing Enzyme CYP26B1 and the Development of Crohn's Disease. *Plos One* 2013; 8.
 - 60 Sanders TJ, McCarthy NE, Giles EM, Davidson KLM, Haltalli MLR, Hazell S et al. Increased Production of Retinoic Acid by Intestinal Macrophages Contributes to Their Inflammatory Phenotype in Patients With Crohn's Disease. *Gastroenterology* 2014; 146: 1278-+.
 - 61 Takayama T, Kamada N, Chinen H, Okamoto S, Kitazume MT, Chang J et al. Imbalance of NKp44(+) NKp46(-) and NKp44(-)NKp46(+) Natural Killer Cells in the Intestinal Mucosa of Patients With Crohn's Disease. *Gastroenterology* 2010; 139: 882-U251.
 - 62 Wolk K, Witte E, Hoffmann U, Doecke WD, Endesfelder S, Asadullah K et al. IL-22 Induces Lipopolysaccharide-Binding Protein in Hepatocytes: A Potential Systemic Role of IL-22 in Crohn's Disease. *The Journal of Immunology* 2007; 178: 5973-81.
 - 63 Burkitt DP. Epidemiology of Cancer of Colon and Rectum. *Cancer* 1971; 28: 3-&.
 - 64 Ben QW, Sun YW, Chai R, Qian AH, Xu B, Yuan YZ. Dietary Fiber Intake Reduces Risk for Colorectal Adenoma: A Meta-analysis. *Gastroenterology* 2014; 146: 689-+.
 - 65 Figueiredo JC, Hsu L, Hutter CM, Lin Y, Campbell PT, Baron JA et al. Genome-Wide Diet-Gene Interaction Analyses for Risk of Colorectal Cancer. *Plos Genetics* 2014; 10.
 - 66 Bordonaro M, Lazarova DL, Sartorelli AC. Butyrate and Wnt signaling - A possible solution to the puzzle of dietary fiber and colon cancer risk? *Cell Cycle* 2008; 7: 1178-83.
 - 67 Lazarova DL, Chiaro C, Wong T, Drago E, Rainey A, O'Malley S et al. CBP Activity Mediates Effects of the Histone Deacetylase Inhibitor Butyrate on WNT Activity and Apoptosis in Colon Cancer Cells. *Journal of Cancer* 2013; 4: 481-90.
 - 68 Lazarova D, Chiaro C, Bordonaro M. Butyrate induced changes in Wnt-signaling specific gene expression in colorectal cancer cells. *BMC Research Notes* 2014; 7: 226.
 - 69 Ou JH, Carbonero F, Zoetendal EG, Delany JP, Wang M, Newton K et al. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *American Journal of Clinical Nutrition* 2013; 98: 111-20.
 - 70 Fechner A, Fenske K, Jahreis G. Effects of legume kernel fibres and citrus fibre on putative risk factors for colorectal cancer: a randomised, double-blind, crossover human intervention trial. *Nutrition Journal* 2013; 12.
 - 71 Takahashi-Yanaga F, Kahn M. Targeting Wnt Signaling: Can We Safely Eradicate Cancer Stem Cells? *Clinical Cancer Research* 2010; 16: 3153-62.
 - 72 Huawei Zeng, Darina L Lazarova, Michael Bordonaro. Mechanisms linking dietary fiber, gut microbiota and colon cancer prevention. *World J Gastrointest Oncol.* 2014; 6: 41-51.
 - 73 Schulz MD, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature* 2014; advance online publication.
 - 74 Brown GT, Cash BG, Blihoghe D, Johansson P, Alnabulsi A, Murray GI. The Expression and Prognostic Significance of Retinoic Acid Metabolising Enzymes in Colorectal Cancer. *Plos One* 2014; 9.
 - 75 Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013; 498: 99-+.
 - 76 Qin JJ, Li YR, Cai ZM, Li SH, Zhu JF, Zhang F et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; 490: 55-60.
 - 77 Wang X, Ota N, Manzanillo P, Kates L, Zavala-Solorio J, Eidenschenk C et al. Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. *Nature* 2014; advance online publication.
 - 78 Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism-Clinical and Experimental* 2012; 61: 1058-66.
 - 79 Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, Mccue T et al. Microbiota Modulate Behavioral

-
- and Physiological Abnormalities Associated with Neurodevelopmental Disorders. *Cell* 2013; 155: 1451-63.
- 80 Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren BY et al. The Treatment-Naive Microbiome in New-Onset Crohn's Disease. *Cell Host & Microbe* 2014; 15: 382-92.
- 81 Simren M, Barbara G, Flint HJ, Spiegel BMR, Spiller RC, Vanner S et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut* 2013; 62: 159-76.
- 82 Whelan K, Quigley EMM. Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease. *Current Opinion in Gastroenterology* 2013; 29: 184-9.
- 83 Cammarota G, Ianiro G, Bibbo S, Gasbarrini A. Gut microbiota modulation: probiotics, antibiotics or fecal microbiota transplantation? *Internal and Emergency Medicine* 2014; 9: 365-73.
- 84 Ubeda C, Pamer EG. Antibiotics, microbiota, and immune defense. *Trends in Immunology* 2012; 33: 459-66.
- 85 Smits LP, Bouter KEC, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic Potential of Fecal Microbiota Transplantation. *Gastroenterology* 2013; 145: 946-53.
- 86 King DE, Mainous AG, Lambourne CA. Trends in Dietary Fiber intake in the United States, 1999-2008. *Journal of the Academy of Nutrition and Dietetics* 2012; 112: 642-8.
- 87 Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. *Journal of Gastroenterology* 2014; 49: 785-98.