REVIEW

Intestinal RORγt-generated Th17 cells control type 2 diabetes: A first antidiabetic target identified from the host to microbiota crosstalk

Céline Pomié[1,2,*], Lucile Garidou[1,2,*], Rémy Burcelin[1,2]

[1]*Institut des Maladies Métaboliques et Cardiovasculaires, INSERM U1048 F-31432 Toulouse, France*
[2]*Université Paul Sabatier, F-31432 Toulouse, France*

[*]Authors contributed equally to this work.
Correspondence: Rémy Burcelin
E-mail: remy.burcelin@inserm.fr
Received: October 20, 2015
Published online: August 04, 2016

The recent discovery of the role played by gut microbiota on the control of metabolic disease opens novel routes for the identification of the causes of type 2 diabetes and obesity. This paradigm could explain the infiltration, by innate and adaptive immune cells, of the adipose tissue, liver, and islets of Langerhans which is responsible for the metabolic inflammation state that leads to impaired insulin action and secretion, and therefore, type 2 diabetes. The identification of the causal role of circulating lipopolysaccharides (LPS) and peptidoglycans in the development of metabolic inflammation, due to an increased intestinal permeability, led to the leaky gut hypothesis. In addition, whole live bacteria were found in metabolic tissues establishing a tissue microbiota which upon a fat-enriched diet becomes dysbiotic. The process of intestinal bacterial translocation was responsible for the onset of a leaky gut causal to the disease. The translocation of selective sets of intestinal bacteria to the blood could be identified. These blood bacterial 16SrRNA-DNA sequences are considered as biomarkers of the bacterial translocation process. An increased of the corresponding bacterial DNA concentration was predicting the occurrence of type 2 diabetes. Associated to the dysbiotic microbiota translocation, an impaired intestinal immune defense was identified as a cause of the selective leaky gut. The change in small intestine mucosal microbiota induced by a fat-enriched diet reduces the number of IL17-secreting CD4 T cells within the lamina propria of the intestine. This loss of IL17-secreting CD4 T cells is the consequence of an impaired capacity of intestinal antigen presenting cells to activate and trigger the expression of Retineic-acid-receptor-related orphan nuclear receptor gamma (RORγt) and the production of IL17 by CD4 T cells. Altogether, an impaired intestinal immune defense, notably the reduced differentiation of RORγt expressing IL17-producing CD4 T cells, favors the onset of a leaky gut leading to the translocation of bacterial factors and live bacteria towards tissues triggering metabolic inflammation; insulin resistance and type 2 diabetes. Hence, the triggering of intestinal defense surrounding RORγt pathway now appears as a potential target mechanism for the control of type 2 diabetes.

Keywords: Microbiota; metabolic disease; intestinal immunity; Th17; diabetes; obesity; germ-free/axenic mice

Abbreviations: LPS, lipopolysaccharides; RORγt, Retineic-acid-receptor-related orphan nuclear receptor gamma; IL, Interleukins; ILC3, innate lymphoid cells type 3; SFB, Segmentous Filamentous Bacteria

To cite this article: Céline Pomié, *et al*. Intestinal RORγt-generated Th17 cells control type 2 diabetes: A first antidiabetic target identified from the host to microbiota crosstalk. Inflamm Cell Signal 2016; 3: e1074. doi: 10.14800/ics.1074.

The last decade has demonstrated the importance of gut microbiota on the control of metabolic diseases through mechanisms that are currently been uncovered. More precisely, this relationship can explain the key deleterious role of metabolic inflammation described in insulin sensitive tissues such as the liver, adipose depots and muscles but can also explain the epidemic development of the disease. In 2025 more than 350 million people will become type 2 diabetic with a doubling of this incidence in oriental countries. Hence, a "contagious" agent was suspected to explain such epidemic development. The commensal bacterial ecology from the gut was demonstrated to be dysbiotic in obese and type 2 diabetic humans and rodents and was representing a leading hypothesis to the epidemic development.

The gut microbiota encompasses trillions of bacteria which are mostly [>90%] represented by two phyla, the *Bacteroidetes* and the *Firmicutes*. *Actinobacteria* and *Proteobacteria*, although minor, are also important phyla that play a role in the ecology of gut microbiota and its relationship with the host. Bacteria, along the digestive track are distributed according to gradients of pH and oxygen. Taxa able to live in a low pH and high pressure of oxygen are present starting from the stomach until the distal ileum, while at the entry into the proximal colon most of the microbial ecology is composed of anaerobic and fermenting bacteria. Another major player responsible for the control of gut microbiota ecology is the intestinal defense system which includes first, a mucus layer composed of mucin, proteins which gets thicker and sealer, as the microbiota develops along the intestine from the proximal to the distal intestinal track. Second, antimicrobial peptides are secreted by Paneth cells, i.e. defensins, which are natural antibiotics that eliminate bacteria from the mucus layer, which would not be allowed to survive close or within the mucus. Third, the immune system, including both the innate and the adaptive immune systems, shapes gut microbiota. The innate immune cells, including dendritic cells and macrophages, sample bacteria from the mucus layer and those that succeed in translocating from the lumen to the lamina propria of the villi. Bacterial antigen presentation by these innate immune cells leads to the activation of T lymphocytes. In addition, antibody-secretion specialized B cells, plasmocytes produce mg per day of immunoglobulins which are secreted within the intestine and targeted the gut microbiota. Altogether, the gut microbiota inherited mostly at birth from the mother and close relatives stabilizes around 2-3 years of age and remains under a symbiotic control with the host up until aging. Importantly, it is conceivable that the bacteria present in the upper digestive track have different metabolic features and therefore functions, when compared to those present in the colon. A major issue concerns the role played by the microbiota from the different intestinal segments on the control of energy metabolism. In physiological situations, the gut microbiota ensures digestive functions throughout the hydrolysis and fermentation of non-digestible nutrients such as dietary fibers into proprionic, butyric and lactic acids. The colonic fermenting bacteria are mostly handling this function. Gut microbiota also contributes to lipid emulsification through the production of dehydroxylated, sulfated or nitrosylated bile acids, the latters being important for the regulation of energy metabolism. Eventually, gut microbiota is also responsible for the synthesis of vitamins such as the B12. The overall role of gut microbiota is to induce the development of important functions and among them digestion and immunity.

A dysbiotic gut microbiota was markedly observed in patients with metabolic diseases such as obesity and type 2 diabetes. It is characterized by changes in the proportion of Firmicutes and Bacteriodetes along with an increased proportion of the Proteobacteria. Microbiota dysbiosis was demonstrated to be causally involved in the development of the disease. The colonization of a germ free mouse with the gut microbiota from a mouse in which metabolic disease and gut microbiota dysbiosis have been induced by a fat-enriched diet induced metabolic disease [1]. Ever since, physiological mechanisms were proposed which could explain the role played by gut microbiota. Among them, the control of bile acids [2], GLP-1 secretion [3], intestinal gluconeogenesis [4] or intestinal inflammation [5], are the most promising. However, out of these hypotheses, none could causally reconcile gut microbiota dysbiosis with metabolic inflammation i.e. tissue inflammation, and insulin resistance. A further mechanism was required. In 2007, we identified that increased plasma LPS concentrations, i.e. metabolic endotoxemia, was responsible for high-fat diet-induced metabolic inflammation leading to metabolic disease [6]. This mechanism was demonstrated by the mean of low rates of LPS infusion which triggered within a month glucose intolerance, hepatic insulin resistance, hepatic steatosis, and adipose tissue development while mice deleted for the LPS receptor, i.e. CD14, resisted the occurrence of metabolic diseases. We further showed that LPS could causally trigger the proliferation of adipocyte precursors and macrophages [7] through a mechanism requiring CD14 to detect the LPS moieties. More recently, we identified that bacterial 16SrRNA-DNA was also detected in a higher concentration in apparently healthy individuals which were predicted to become type 2 diabetic within the next 6-9 years [8]. A doubling of the 16SrRNA-DNA concentration significantly increased the incidence of type 2 diabetes. The sequencing of the 16SrRNA-DNA gene demonstrates that most of the bacterial DNA was from the *Proteobacteria* family which are LPS producing bacteria. Numerous mechanisms are

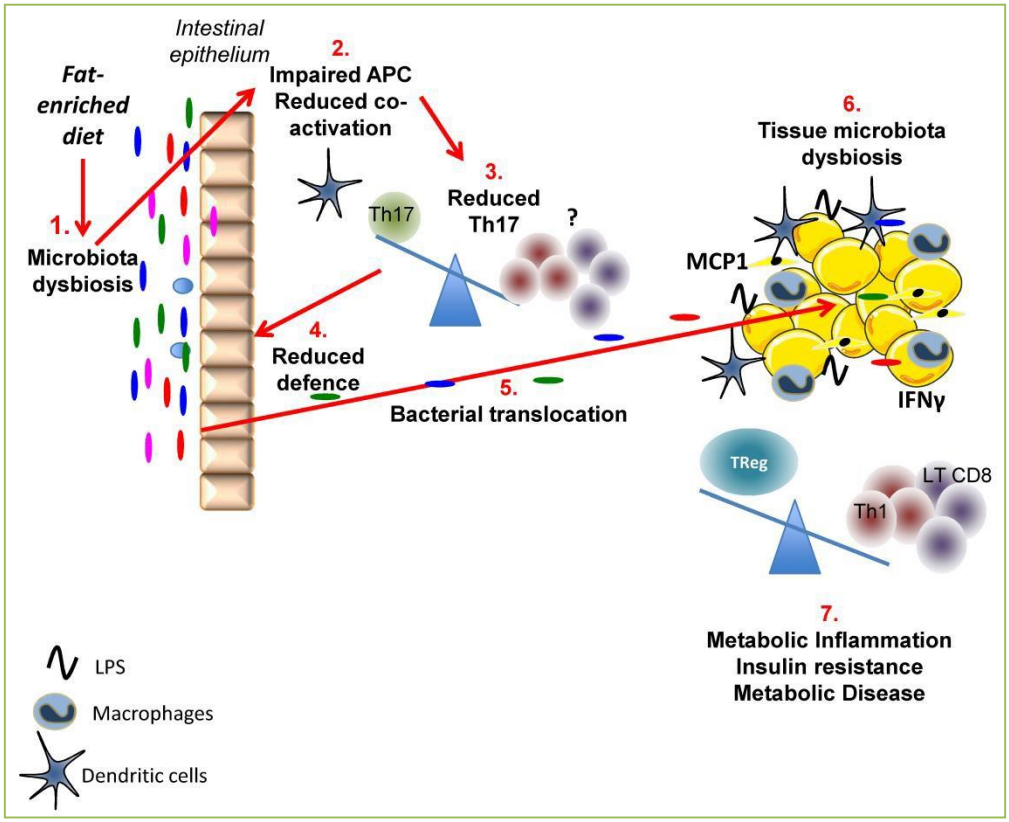

Figure 1. Targeting intestinal RORγτ/IL17 producing CD4 T cells to control metabolic disease. Gut microbiota dysbiosis induced by a high-fat diet impairs the capacity of the intestinal antigen presenting cells to induce the differentiation of IL17 producing CD4 T cells. This decrease in intestinal Th17 cells reduces the intestinal defense, in part through a decrease in the production of antimicrobial peptides, allowing an increase in bacterial translocation from intestine towards metabolic tissues. Bacterial determinants such as LPS, initiates the activation of immune system in metabolic tissues so that the production of inflammatory chemokines and cytokines trigger insulin resistance and to the development of metabolic disease. Targeting intestinal defense could be considered as a novel source of therapeutic perspectives.

responsible for metabolic endotoxemia. Among them an increased intestinal permeability [9] and a change in LPS clearance are linked to a lowering of the circulating lipoprotein metabolism [10]. The increased bacterial DNA concentration in blood was linked to an increased translocation of intestinal bacteria towards the tissues including the blood through mechanisms [11] requiring and impaired intestinal recognition of bacterial determinants such as LPS and peptidoglycans by NOD1/2 and TLR4/CD14 receptors [12, 13]. Bacterial translocation is a naturally occurring mechanism that in some instances is dramatically increased such as in patients with HIV [14] or leaky gut [11, 13, 15]. Altogether, an impaired intestinal defense in type 2 diabetic patients was suggested to be responsible for the increased bacterial translocation, the establishment of a dysbiotic tissue microbiota [16] and hence an increased metabolic inflammation and the development of metabolic diseases.

Recent data from the literature identified that tissue metabolic inflammation involved innate [17, 18] and adaptive immune systems [19, 20]. First, metabolic diseases were largely characterized by an infiltration of metabolic tissue such as adipose tissue by macrophages. Limiting the infiltration of macrophages in adipose tissue reduced the inflammatory profile and improved insulin sensitivity [17, 21, 22]. Some data also argued for a role of dendritic cells in the initiation or the maintenance of metabolic inflammation since the loss of weight reduced the number of infiltrating cells along with a decrease of pro-inflammatory adipokines in obese patients [23; 24]. Ablation of dendritic cells in insulin-resistant mice, decreased inflammatory markers and normalized insulin sensitivity [25]. In addition, other innate immune cells, such as mast cells, infiltrated adipose tissue during metabolic disease [26] where these cells, through their MHCII, could present antigens and activate T lymphocytes. An important role of T lymphocytes in the development of metabolic disease was also demonstrated with the accumulation of inflammatory CD8 T lymphocytes in adipose tissue of obese mice [27]. Ablation of CD8 T lymphocytes decreased the adipose tissue

macrophages infiltration and improved metabolic parameters. Conversely, their adoptive transfer aggravated adipose inflammation while contrarily to CD8 T lymphocytes, the number of regulatory Foxp3+ CD4 T lymphocytes was decreased in adipose tissue of obese and insulin-resistant mice [28]. Transfer of regulatory T lymphocytes improved metabolic inflammation and insulin sensitivity by toning down the overall metabolic inflammatory process demonstrating the importance of not only innate immune cells but also of the adaptive immune system. Others lymphocytes might also be involved in the maintenance of metabolic inflammation such as NKT which recognize lipids in the context of CD1d [29] and B lymphocytes that the concentration is also increased in metabolic tissues following diet-induced obesity [30]. Altogether, activation, recruitment and infiltration of immune cells, within metabolic tissues, adipose depots and liver, lead to the secretion of immune factors such as cytokines that maintain a low-grade inflammatory tone and are deleterious for cellular insulin signaling leading to an impaired insulin action.

However, the origin of the determinants responsible for the activation of the tissue immune system remained unclear. We hypothesized that the bacterial translocation, through the establishment of the tissue microbiota could be responsible [16, 31]. Therefore, features from the intestinal immune defense must have been altered in conditions of gut microbiota dysbiosis such as in response to a fat-enriched diet. We explored this hypothesis and identified changes in intestinal immunity before the onset of insulin resistance. A decrease in the number of inflammatory IL17-producing CD4 T cells was observed in the lamina propria of the ileum after only 10 days of a fat-enriched diet and was maintained and even exacerbated after 30 days. Since RORγt is the key transcription factor responsible for the differentiation of naïve CD4 T lymphocytes into Th17/22 differentiated lymphocytes, we quantified the RORγt-expressing CD4 T lymphocytes and found them similarly reduced in the lamina propria of the high fat diet-fed mice after only 10 days. The corresponding deficient mice, i.e. without challenging them with a high fat diet, developed spontaneously insulin resistance, glucose intolerance and other metabolic features demonstrating the importance of this gene on the control of energy metabolism. The transfer of T cells isolated from RORγt deficient mice into Rag1 deficient mice induced the development of metabolic disease, demonstrating the causal role of Th17 cells. This experiment demonstrated that an impaired Th17 cell production was sufficient to induce metabolic disease. However, we cannot rule out that other IL17-producing cells such as innate lymphoid cells type 3 (ILC3) have no role. Few weeks after this transfer, mice spontaneously developed glucose intolerance and insulin resistance, which clearly demonstrated a pivotal protecting role for Th17 cells in the control of metabolic disease. In agreement with our results, a positive role for intestinal IL22, another cytokine produced in part by Th17 cells, was recently demonstrated for the control of metabolic disorders [32]. Furthermore, in HIV-infected human patients, who have a higher incidence of type 2 diabetes [33], a loss of IL22 producing CD4 T cells was reported [34] and was associated with an increased bacterial translocation [14].

Since metabolic disease is associated with gut microbiota dysbiosis and since the adaptive and innate immune systems are in closed contact and influenced by microbiota [35], we speculated that changes in gut microbiota induced by a fat-enriched diet would be responsible for the loss of intestinal IL17-producing cells. To causally incriminate gut microbiota dysbiosis, high-fat diet-fed mice were orally treated with a mixture of prebiotic and probiotic (synbiotic) known to modify gut microbiota dysbiosis and to improve high-fat diet-induced metabolic disease [36]. We first validated that a one-month synbiotic treatment drastically changed the ileum microbiota. We focused our attention on the ileum microbiota since the major drastic changes of the number of IL17-producing CD4 T cells observed were within the lamina propria of this intestinal segment. Furthermore, this is an intestinal location where images of bacterial translocation were clearly observed [13]. We observed that the synbiotic treatment prevented the loss of intestinal Th17 and the development of high fat diet-induced metabolic disease. The dysbiotic microbiota was characterized by a reduction in the proportion of *Porphyromonadaceae* that was totally normalized by the synbiotic treatment along with the improvement of all metabolic parameters. The *Porphyromonadaceae* are known to promote Th17 inducing pathways [37] as observed during periodontitis [38]. Therefore, their reduced frequency within the intestine could be linked to the reduced number of IL17 producing cells within the lamina propria of the small intestine. *Segmentous Filamentous Bacteria* (SFB) are known to induce, at least in the mouse, the differentiation of Th17 cells [39; 40]. However, although their frequency was reduced by more than 90% in the intestine from prediabetic mice it was not restored by the synbiotic treatment whereas the number of IL17-producing CD4 T cells and the metabolic features were improved. Therefore, a change in SFB frequency was not responsible, in our experiment, for the increased IL17-producing CD4 T cells and the improved metabolic phenotype. We cannot rule out that a therapeutic strategy against metabolic disease could be derived from SFB aiming at restoring intestinal IL17 production. This still needs to be tested. Similarly, it can be suggested that the microbiota from other intestinal segments could be involved as well. Although, we do not have argument supporting this hypothesis, it cannot be ruled out. Then, to clearly demonstrate a causal role of the ileum

microbiota dysbiosis on the decrease number of intestinal Th17 cells, germ free mice were colonized with the microbiota isolated from the ileum of high-fat diet-fed mice treated or not with synbiotic. Conversely to the ileum microbiota from high-fat diet-fed mice, ileum microbiota of symbiotic treated mice increased the number of intestinal Th17 and prevented the development of metabolic disease, showing that bacteria present in synbiotic-treated microbiota could induce intestinal Th17 cells. Interestingly, in addition to microbiota, viruses like bacteriophages could be involved, since the latters are requiring specific bacteria isotype to develop. Therefore, the viruses could be controlling IL17 producing cells, instead of bacteria, while the bacteria will just be required for the growth and production of the active viruses. This is again another hypothesis that needs to be explored. Another demonstration that intestinal Th17 are crucial for the protection against metabolic disease is that induction of experimental colitis, which induced intestinal Th17, prevented the development of metabolic disease. *Peptostreptococcaceae*, another intestinal bacterial family, was reported to be involved in the prevention of the development of colitis. Since we have observed an increase in this bacterial family under a fat enriched diet that is prevented by the symbiotic treatment, *Peptostreptococcaceae* would be a candidate involved in the loss of intestinal Th17 at the onset of metabolic disease.

This complex ecology of bacteria could impact differentiation of intestinal Th17 by controlling antigen presenting cell functions. Transciptomic analysis revealed that high-fat diet-induced microbiota dysbiosis was associated with alteration of antigen presenting cells transcriptomic profiles suggesting an impaired capacity to activate T cells. *In vitro*, intestinal antigen presenting cells isolated from high-fat diet-fed mice were less able to produce cytokines, i.e. IL6 IL12p40 involved in the differentiation and maintenance of Th17 cells, and to promote differentiation of na ïve T cells into Th17. Overall, changes in ileum microbiota [induced by a fat-enriched diet] could impair the ability of intestinal antigen presenting cells to promote Th17. This mechanism could explain the improvement of insulin resistance in human after a bariatric surgery, since this surgery is associated with dramatic changes in gut microbiota [41].

As a consequence of the decrease in IL17 produced by intestinal CD4 T cells, anti-microbial peptides could be altered such as Reg3β and Reg3γ which expression decreased similarly to the loss of intestinal Th17. Alteration of mucosal protection could allow an increase in dysbiotic bacterial translocation towards metabolic tissues where deleterious bacteria could initiate metabolic inflammation. Interestingly, human studies from jejunal samples of obese subjects without comorbidity, or suffering from obesity-related comorbidity, or diabetic, versus lean controls, showed that obesity is associated with T-cell-mediated inflammation and enterocyte insulin resistance [42]. Although, it could not be causally linked to the development of the disease, the authors identified an increased number of both innate and adaptive immune cells particularly CD8αβ T cells in the obese jejunum epithelium in association with an increase in inflammatory cytokines suggesting an intestinal inflammation. Importantly, the authors showed an increased Th17 cells in the lamina propria of obese patients with a mean body mass index over 45, which represents only a small subset of the overall obese patients where BMI are closer to 30-35. These patients could be also considered as late onset where the increased intestinal inflammation and enterocyte insulin resistance could be considered consequences to the disease. Moreover, human data are not available regarding the general obese patient population.

Altogether, a change in gut immunity linked to gut microbiota dysbiosis is now considered as a novel cellular mechanism associated to metabolic disease. The intestine is a major player of energy homeostasis where the molecular crosstalk between intestinal cells and gut microbiota could be considered as a novel source of therapeutic perspectives.

Conflicting interests

The authors have declared that no competing interests exist.

References

1. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-1031.
2. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, *et al*. FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 2014;509:183-188.
3. Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, Reimann F. Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. Cell reports 2014;9:1202-1208.
4. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, *et al*. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 2014;156:84-96.
5. Everard A, Geurts L, Caesar R, Van Hul M, Matamoros S, Duparc T, *et al*. Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status. Nature communications 2014;5:5648.
6. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761-1772.
7. Luche E, Cousin B, Garidou L, Serino M, Waget A, Barreau C, *et*

al. Metabolic endotoxemia directly increases the proliferation of adipocyte precursors at the onset of metabolic diseases through a CD14-dependent mechanism. Mol Metab 2013;2:281-291.

8. Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, *et al*. Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. Diabetologia 2011;54:3055-3061.

9. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, *et al*. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470-1481.

10. Verges B, Duvillard L, Lagrost L, Vachoux C, Garret C, Bouyer K, *et al*. Changes in lipoprotein kinetics associated with type 2 diabetes affect the distribution of lipopolysaccharides among lipoproteins. The Journal of clinical endocrinology and metabolism 2014:jc20133463.

11. Berg RD. Bacterial translocation from the gastrointestinal tract. Trends Microbiol 1995;3:149-154.

12. Schertzer JD, Tamrakar AK, Magalhaes JG, Pereira S, Bilan PJ, Fullerton MD, *et al*. NOD1 activators link innate immunity to insulin resistance. Diabetes 2011;60:2206-2215.

13. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermudez-Humaran LG, *et al*. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med 2011;3:559-572.

14. Mavigner M, Cazabat M, Dubois M, L'Faqihi FE, Requena M, Pasquier C, *et al*. Altered CD4+ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest 2012;122:62-69.

15. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, *et al*. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. J Exp Med 2011;208:125-134.

16. Burcelin R, Serino M, Chabo C, Garidou L, Pomie C, Courtney M, *et al*. Metagenome and metabolism: the tissue microbiota hypothesis. Diabetes, obesity & metabolism 2013;15 Suppl 3:61-70.

17. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, *et al*. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest 2006;116:115-124.

18. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-1808.

19. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, *et al*. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 2009;15:921-929.

20. Cipolletta C, Ryan KE, Hanna EV, Trimble ER. Activation of Peripheral Blood CD14+ Monocytes Occurs in Diabetes. Diabetes 2005;54:2779-2786.

21. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007;56:16-23.

22. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, *et al*.MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 2006;116:1494-1505.

23. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, *et al*. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005;54:2277-2286.

24. Capel F, Klimcakova E, Viguerie N, Roussel B, Vitkova M, Kovacikova M, *et al*. Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization. Diabetes 2009;58:1558-1567.

25. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 2008;8:301-309.

26. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, *et al*. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 2009;15:940-945.

27. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, *et al*. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009;15:914-920.

28. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, *et al*. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009;15:930-939.

29. Ohmura K, Ishimori N, Ohmura Y, Tokuhara S, Nozawa A, Horii S, *et al*. Natural killer T cells are involved in adipose tissues inflammation and glucose intolerance in diet-induced obese mice. Arterioscler Thromb Vasc Biol 2010;30:193-199.

30. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, *et al*. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 2011;17:610-617.

31. Burcelin R, Garidou L, Pomie C. Immuno-microbiota cross and talk: the new paradigm of metabolic diseases. Semin Immunol 2012;24:67-74.

32. 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;514:237-241.

33. Samaras K. The burden of diabetes and hyperlipidemia in treated HIV infection and approaches for cardiometabolic care. Current HIV/AIDS reports 2012;9:206-217.

34. Page EE, Greathead L, Metcalf R, Clark SA, Hart M, Fuchs D, *et al*. Loss of Th22 cells is associated with increased immune activation and IDO-1 activity in HIV-1 infection. Journal of acquired immune deficiency syndromes 2014;67:227-235.

35. Sommer F, Backhed F. The gut microbiota-masters of host development and physiology. Nature reviews Microbiology 2013;

36. Kolida S, Gibson GR. Synbiotics in health and disease. Annual review of food science and technology 2011;2:373-393.

37. Moutsopoulos NM, Kling HM, Angelov N, Jin W, Palmer RJ, Nares S, *et al*. Porphyromonas gingivalis promotes Th17 inducing pathways in chronic periodontitis. Journal of autoimmunity 2012;39:294-303.

38. Wang L, Wang J, Jin Y, Gao H, Lin X. Oral administration of all-trans retinoic acid suppresses experimental periodontitis by modulating the Th17/Treg imbalance. Journal of periodontology 2014;85:740-750.

39. Farkas AM, Panea C, Goto Y, Nakato G, Galan-Diez M, Narushima S, *et al*. Induction of Th17 cells by segmented filamentous bacteria in the murine intestine. J Immunol Methods 2015;421:104-111.

40. Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, *et al*. Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. Cell 2015;163(2):367-380.

41. Aron-Wisnewsky J, Clement K. The effects of gastrointestinal surgery on gut microbiota: potential contribution to improved insulin sensitivity. Curr Atheroscler Rep 2014;16:454.

42. Monteiro-Sepulveda M, Touch S, Mendes-Sa C, Andre S, Poitou C, Allatif O, *et al*. Jejunal T Cell Inflammation in Human Obesity Correlates with Decreased Enterocyte Insulin Signaling. Cell Metab 2015;22:113-124.