Original article

MOLECULAR METABOLISM

Triggering the adaptive immune system with commensal gut bacteria protects against insulin resistance and dysglycemia

Céline Pomié [1,2], Vincent Blasco-Baque [1,2], Pascale Klopp [1,2], Simon Nicolas [1,2], Aurélie Waget [1,2], Pascale Loubières [1,2], Vincent Azalbert [1,2], Anthony Puel [1,2], Frédéric Lopez [4], Cédric Dray [5], Philippe Valet [5], Benjamin Lelouvier [3], Florence Servant [3], Michael Courtney [3], Jacques Amar [1,2], Rémy Burcelin [1,2,*], Lucile Garidou [1,2,**]

ABSTRACT

Objective: To demonstrate that glycemia and insulin resistance are controlled by a mechanism involving the adaptive immune system and gut microbiota crosstalk.

Methods: We triggered the immune system with microbial extracts specifically from the intestinal ileum contents of HFD-diabetic mice by the process of immunization. 35 days later, immunized mice were fed a HFD for up to two months in order to challenge the development of metabolic features. The immune responses were quantified. Eventually, adoptive transfer of immune cells from the microbiota-immunized mice to naïve mice was performed to demonstrate the causality of the microbiota-stimulated adaptive immune system on the development of metabolic disease. The gut microbiota of the immunized HFD-fed mice was characterized in order to demonstrate whether the manipulation of the microbiota to immune system interaction reverses the causal deleterious effect of gut microbiota dysbiosis on metabolic disease.

Results: Subcutaneous injection (immunization procedure) of ileum microbial extracts prevented hyperglycemia and insulin resistance in a dose-dependent manner in response to a HFD. The immunization enhanced the proliferation of CD4 and CD8 T cells in lymphoid organs, also increased cytokine production and antibody secretion. As a mechanism explaining the metabolic improvement, the immunization procedure reversed gut microbiota dysbiosis. Finally, adoptive transfer of immune cells from immunized mice improved metabolic features in response to HFD.

Conclusions: Glycemia and insulin sensitivity can be regulated by triggering the adaptive immunity to microbiota interaction. This reduces the gut microbiota dysbiosis induced by a fat-enriched diet.

Keywords Gut microbiota and metabolic diseases; Immunity; Insulin resistance

1. INTRODUCTION

Trillions of bacteria inhabit our gut establishing a stable ecology and symbiosis with the host. The last decade demonstrated that changes in this ecology characterize metabolic disease [1]. This dysbiosis was reversible since diet-induced body weight loss restored a healthy gut microbiota ecology further suggesting its causal role [2,3]. The causality of gut microbiota during the development of metabolic disease was demonstrated in rodents by showing that the obesity phenotype could be transferred from obese mice to germ free recipient mice by colonizing the latter with the cecal or ileal microbiota from the former [4,5]. It was also shown that hepatic steatosis [6], atherosclerosis [7] as well as the therapeutic effect of bariatric surgery on obesity [8] and the deleterious effect of dietary lipids [9] were under the control, at least in part, of the gut microbiota. Although the causal role of gut microbiota in the control of metabolic disease is commonly accepted, the identification of the corresponding mechanisms is in its infancy. An important leading concept is that gut microbiota ecology regulates the

[1]Institut National de la Santé et de la Recherche Médicale (INSERM), Toulouse, France [2]Université Paul Sabatier (UPS), Unité Mixte de Recherche (UMR) 1048, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Team 2: « Intestinal Risk Factors, Diabetes, Dyslipidemia », 1 avenue Jean Poulhès, BP 84225, F-31432 Toulouse Cedex 4, France [3]VAIOMER SAS, 516 Rue Pierre et Marie Curie, 31670 Labège, France [4]Groupe Protéomique Centre Recherche Cancer Toulouse, Université Paul Sabatier (UPS), Unité Mixte de Recherche (UMR) 1037, 2 avenue Hubert Curien, CS 53717, 31037 Toulouse Cedex 1, France [5]Université Paul Sabatier (UPS), Unité Mixte de Recherche (UMR) 1048, Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Team 3: « Adipokines, obesity and associated Pathologies », 1 avenue Jean Poulhès, BP 84225, F-31432 Toulouse Cedex 4, France

*Corresponding author. INSERM UMR1048-I2MC, Team 2 "Intestinal Risk Factors, Diabetes and Dyslipidemia", 1 avenue Jean Poulhès, BP84225, F-31432 Toulouse Cedex 4, France. Tel. +33 561 325 614; fax: +33 5 31 22 41 36. E-mail: remy.burcelin@inserm.fr (R. Burcelin).

**Corresponding author. INSERM UMR1048-I2MC, Team 2 "Intestinal Risk Factors, Diabetes and Dyslipidemia", 1 avenue Jean Poulhès, BP84225, F-31432 Toulouse Cedex 4, France. Tel. +33 561 325 614; fax: +33 5 31 22 41 36. E-mail: lucile.garidou@gmail.com (L. Garidou).

Abbreviations: AT, Adipose tissue; APC, Antigen presenting cells; LN, Lymph nodes; NC, Normal chow; T2D, Type 2 diabetes; VL, Vastus lateralis muscle

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innate and adaptive immune systems at birth [10,11]. Commensal bacteria, such as segmented filamentous bacteria, favor the development of Th17 cells in the gut of rodents [12,13], and *Clostridium* and *Bacteroides fragilis* favor the development of Tregs through mechanisms involving, at least in part, butyrate production from dietary fiber fermentation [14,15]. Alternatively, commensal bacteria overgrowth is under the control of the host immune system, specifically IgAs. These antibodies maintain intestinal vigilance [16] and shape gut microbiota [17]. Consequently, mutations in the immune system are associated with changes in gut microbiota ecology that increase susceptibility to pathologies [18,19]. As an example in the context of metabolic disease, the genetic ablation of the TLR2 gene in mice induced obesity and insulin resistance through modulation of gut microbiota [20]. Importantly, the ecology of gut microbiota varies according to the intestinal segments notably upon the gradient of oxygen, pH, or bile acids [21]. Furthermore, the luminal, mucosal, and cryptic microbiota are different, calling into question the importance of the local dysbiosis. Therefore, the role played by gut microbiota in the control of metabolic disease could depend on a local dysbiosis. Similarly, the intestinal immune system varies according to the intestinal segment [16], and the bacteria interacting with the immune system could be distributed locally rather than widely throughout the gut. Therefore, along the same line of investigation we recently showed that an impaired intestinal defense was characterized by a reduction of the number and frequency of IL17-producing CD4 T cells in the *lamina propria* of the ileum while little or no change was observed in the colon [5].

Thus, the crosstalk between the immune system and gut microbiota localized to the ileum could be crucial in the development of metabolic disease. To address this question, we challenged the immune system with gut ileum microbiota extracts from diabetic mice by an immunization procedure and investigated whether it improved metabolic disease by challenging the mice with a high-fat diet (HFD). The immunization prevents HFD-induced insulin resistance and dysglycemia. The impact on the metabolic features was linked to a mechanism controlling gut microbiota dysbiosis, suggesting that vaccination may be considered as a possible therapeutic approach for T2D.

2. MATERIALS AND METHODS

2.1. Animal models

Eight-week-old C57Bl6/J and CD45.1 C57Bl6/J male mice were purchased from Charles River and maintained at the Inserm Toulouse animal facilities. Rag1-deficient mice were bred in the Inserm Toulouse animal facility. Mice were fed a normal chow diet containing 83.9% cereals, 12% protein, 4.1% vitamin and mineral mixture (Safe, R04) or a HFD containing 72% fat (corn oil, lard), 28% protein, $<1\%$ carbohydrate (Safe). This animal model is intended for studying the impact of a fat-enriched diet independent of obesity. The high content in fat and the absence of carbohydrate favor a rapid hyperglycemia without body weight gain, as described previously [5,22,23]. These mice are moderately insulin resistant, which may be linked to the short duration of the treatment (one month). The HFD is highly lipotoxic and hampers glucose-induced insulin secretion leading to low insulin secretion rates and to hyperglycemia. Linked to the low insulin levels, the body weight of the 70% fat-enriched diet fed mice remains low and could be considered as a MODY like type of model. As an example GK (Goto Kakizaki) rats are largely used in the literature and are lean with low plasma insulin levels. Hence, this diet is a useful tool for studying the impact of a therapeutic strategy on hyperglycemia without the confounding of improved fat mass. All mice were housed under specific pathogen-free conditions. The ethics committee of Rangueil Hospital approved the experiments.

2.2. Immunization of mice

Ten cm-long segments of ileum (up to the cecum) were dissected from mice fed a HFD for 4 weeks. Luminal intestinal contents were collected, suspended in PBS and debris was removed. The supernatant was sonicated (Branson Sonifier 150, Branson Ultrasonics) and diluted in PBS before subcutaneous injection (200 μl). Intestinal extracts were also prepared from mice treated with 1.0 g/l ampicillin and 0.5 g/l neomycin (Sigma) in their drinking water for 4 weeks [24].

2.3. Metabolic parameter monitoring

For the intraperitoneal glucose tolerance test (IPGTT), mice were fasted for 6 h then injected with glucose (1 g/kg, 20% glucose). Glycemia in blood samples collected from the tail vein was determined by means of a glucose meter (Roche Diagnostics). The glycemic index is the sum of the glycemic values multiplied by the duration of the test. Plasma insulin concentration was determined by ELISA (Mercodia Kit) according to the manufacturer's instructions on blood samples collected 30 min before and 15 min after the glucose load. Euglycemic hyperinsulinemic clamping was performed as described previously [25]. Briefly, the mice were fasted for 6 h and then infused at a rate of 18 mU kg^{-1} min^{-1} of insulin for 3 h. Simultaneously, a 20% glucose solution was infused to maintain a steady glycemia while a continuous ^{3}H-glucose was performed to assess the glucose turnover rate. Body mass composition was analyzed by quantitative nuclear magnetic resonance using an EchoMRI-100TM.

2.4. Liver leukocyte isolation

Infiltrating leukocytes were isolated from livers as previously described [26]. Briefly, livers were infused with Liberase (Roche) and incubated for 30 min at 37 °C. Single-cell suspensions were prepared by mechanically disrupting the infused tissue by passing it through a 100 μm filter and then centrifuged. Cells were resuspended in 35% Percoll (Amersham Biosciences). The mononuclear cells were extracted, washed and resuspended in PBS. The number of mononuclear cells was determined.

2.5. Intestinal leukocyte isolation

Small intestine segments (20 cm before the cecum) were harvested, cut into 4 segments (5 cm) and Peyer's patches were dissected out from the intestinal segments. The intestinal segments were incubated in RPMI (Life Technologies) supplemented with EDTA (2 mM, Sigma) three times during 20 min at 37 °C. Segments were then washed with RPMI. Subsequently, the intestinal fragments were incubated in RPMI containing collagenase VIII (0.15 mg/ml; Sigma) and DNase I (40 U/ml; Roche) to isolate *lamina propria* leukocytes. Then leukocytes were isolated through a Percoll gradient (GE Healthcare). The leukocytes were then harvested from the 70%–40% Percoll interface, washed in RPMI and suspended in cell culture medium [5]. The number of mononuclear cells was determined and used to calculate the absolute number.

2.6. Adoptive transfer

Splenocytes from CD45.1 mice were harvested 35 days after immunization and 3×10^{7} cells were injected into the peritoneal cavity of mice fed a HFD for 3 days.

