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Reverse phenotype transfer via fecal microbial transplantation in inflammatory bowel disease

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ABSTRACT

Inflammatory bowel disease (IBD) is characterized by a disbalance in the composition of intestinal microbiota. It is not clear whether such dysbiosis is a cause or a consequence of a disease state. Fecal microbiota transplantation (FMT) from a healthy donor to a patient or diseased animal is a valuable tool for targeted modification of microbiome leading to therapeutic response. Positive effect has been shown in therapy of a number of gastrointestinal as well as non-gastrointestinal diseases. In addition, FMT has been successfully used to transfer the diseased phenotype form a donor with the disease to a healthy recipient. However, targeted modification of the microbiome before the onset of colitis has not been shown previously. Based on our preliminary results, we propose the hypothesis of so called reverse phenotype transfer in IBD. This term describes the phenomenon, in which the transplantation of gut microbiota from a donor more sensitive to IBD to a healthy recipient leads to resistance of the recipient to IBD and vice versa. Mice that received FMT from donors with severe colitis have shown improved colitis score compared with mice that received FMT from donors more resistant to development of colitis. Such reverse phenotype transfer has broad implications, especially in terms of preventive medicine. However, detailed mechanisms need to be elucidated to conclude the validity of the phenomenon.

Background

Inflammatory bowel disease (IBD) is a group of chronic diseases including ulcerative colitis and Crohn's disease. However, pathogenesis is still not completely understood. Multiple gene variants, environmental factors, dysregulated immune system, and gut microbiota have been implicated in initiation and progression of inflammatory state. Gut microbiota has been identified as a new object of interest in pathogenesis of IBD (1).

Patients with IBD have characteristic composition of intestinal microbiota, which is different from healthy individuals [1]. IBD patients have decreased diversity due to shift in the gut microbiota composition with increase in potentially pathogenic bacteria. This results in lower abundances of *Firmicutes* phylum to which belong bacteria such as *Faecalibacterium prausnitzii* that show beneficial properties to its host. On the other hand, Proteobacteria phylum, including *Enterobacteriaceae,* is more abundant and includes potentially harmful species that are able to perpetuate inflammation. Recently, microbial profiles at various stages of colitis have been described and characterized that depend on the time and location within the gastrointestinal tract [2]. It is still unclear whether these changes are simple response to the inflamed state or are the trigger for developing IBD [3,4]. Other microorganisms (fungi, bacteriophages, archaea, eukaryotes) than bacteria must also be taken into consideration when talking about gut microbiome. Changes and possible implications of these microorganisms in IBD pathogenesis are far less studied [5].

Gut microbiome can be therapeutically modulated via several approaches, including antibiotics, probiotics or fecal microbiota transplantation (FMT). FMT represents the transfer of the donor gut microbiota into the recipient with the intention to transplant the donor's phenotype. This approach is superior to other gut microbiota modulations because it transfers the whole consortium of microorganisms, not just several selected species. Several studies have shown that this method is indeed a viable strategy in elucidating the role of gut microbiota [6–8]. Moreover, some studies have shown that IBD can be treated with FMT both in experimental and clinical conditions [9,10]. On the other hand, it is also possible to transplant the disease or at least the predisposition to developing the disease via FMT [11]. However, no studies have shown whether there is a specific microbiota that could protect from developing the disease – "the protective microbiota".

However, from animal experiments it is known that the presence of specifically altered (procolitic) intestinal microbiota has a direct correlation with the development of colitis-associated cancer – CAC [12]. Such targeted change of microbiota (dysbiosis) leading to an increased

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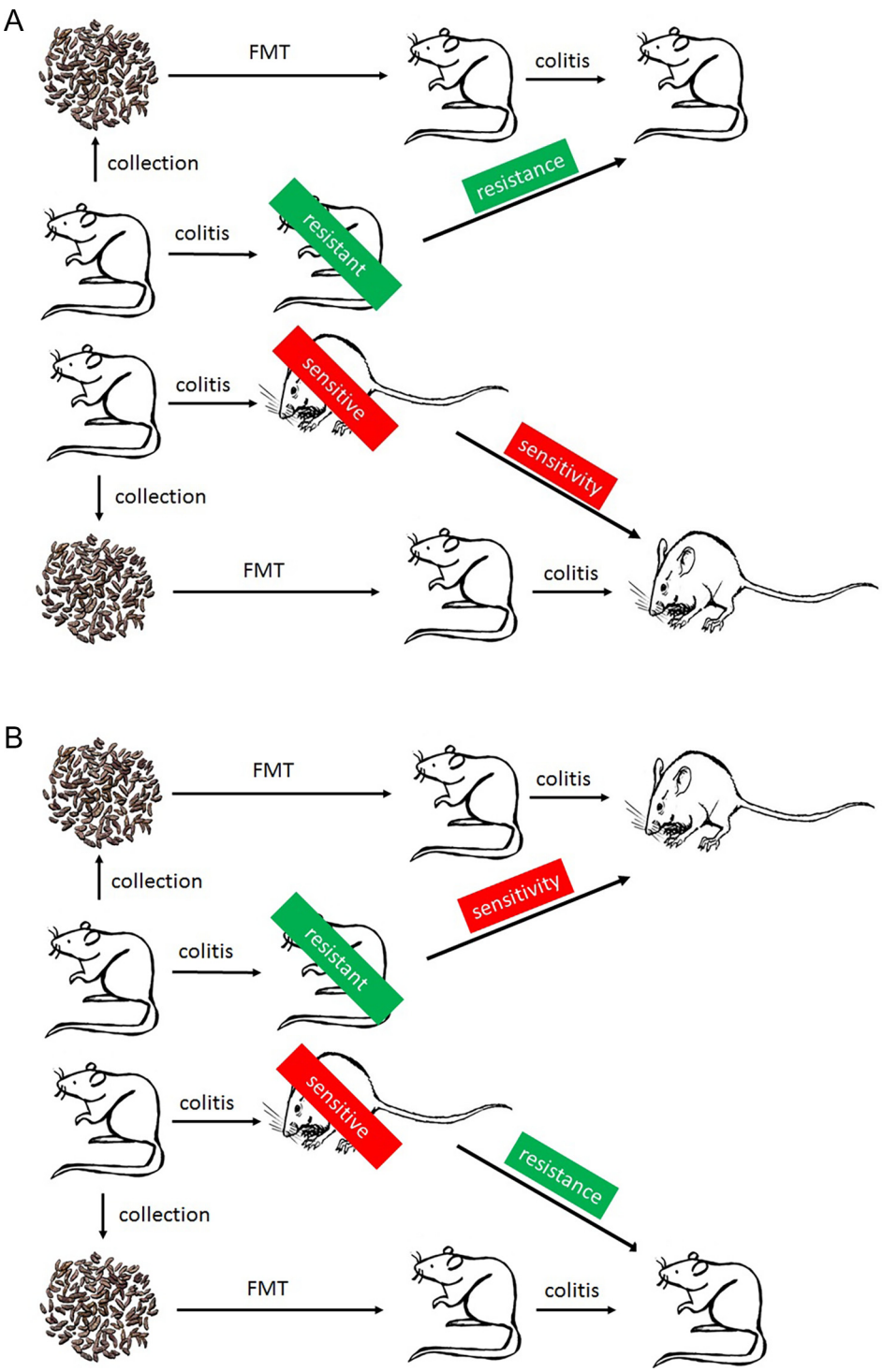

Fig. 1. The anticipated and the observed outcome. A: Anticipated results. Feces from healthy donor mice were frozen for later FMT. Afterwards, chemical colitis was induced in donor mice. Based on colitis severity score, the donor mice were divided into the resistant and the sensitive. The stool collected before induction of colitis was transplanted into healthy recipient mice, which were subsequently induced chemical colitis. The expected outcome was that the recipient mice that received "resistant" microbiota will appear more resistant to disease compared to recipient mice that received sensitive transplant. B: Observed results. The recipient mice that received "resistant" microbiota appeared more sensitive to disease compared to recipient mice that received sensitive transplant. The observed outcome represents the hypothesis of reverse phenotype transfer.

risk of colitis, is reversible and transferable to another individual [11]. Moreover, some results suggest that the mere intestinal tumorigenesis mediated by bacterial dysbiosis may be transferable through the microbiota among individuals with a genetic predisposition [13].

Hypothesis

The proposed hypothesis lies in the so-called "reverse phenotype transfer" via preemptive FMT. Current knowledge says that FMT can be used to transfer the phenotype in a direct way, i.e. either from healthy subject to a patient/diseased animal to transplant the healthy phenotype or from a diseased subject to a healthy one to transplant the pathology. The hypothesis of reverse phenotype transfer is based on a premise that FMT from a healthy donor who is more susceptible to develop disease (sensitive donor) to a healthy recipient will lead to less severe disease in this recipient (resistant recipient) after the induction of the disease.

The idea has evolved from a question whether a specific microbiota exists that protects the subject against the development of IBD and whether this microbiota can be transferred via FMT to another host along with the protective phenotype. Finding an answer to this question might provide valuable data that would contribute to elucidate the pathogenesis of IBD and its possible treatment or prevention. To test this idea, it is necessary to transfer the fecal microbiota from subjects

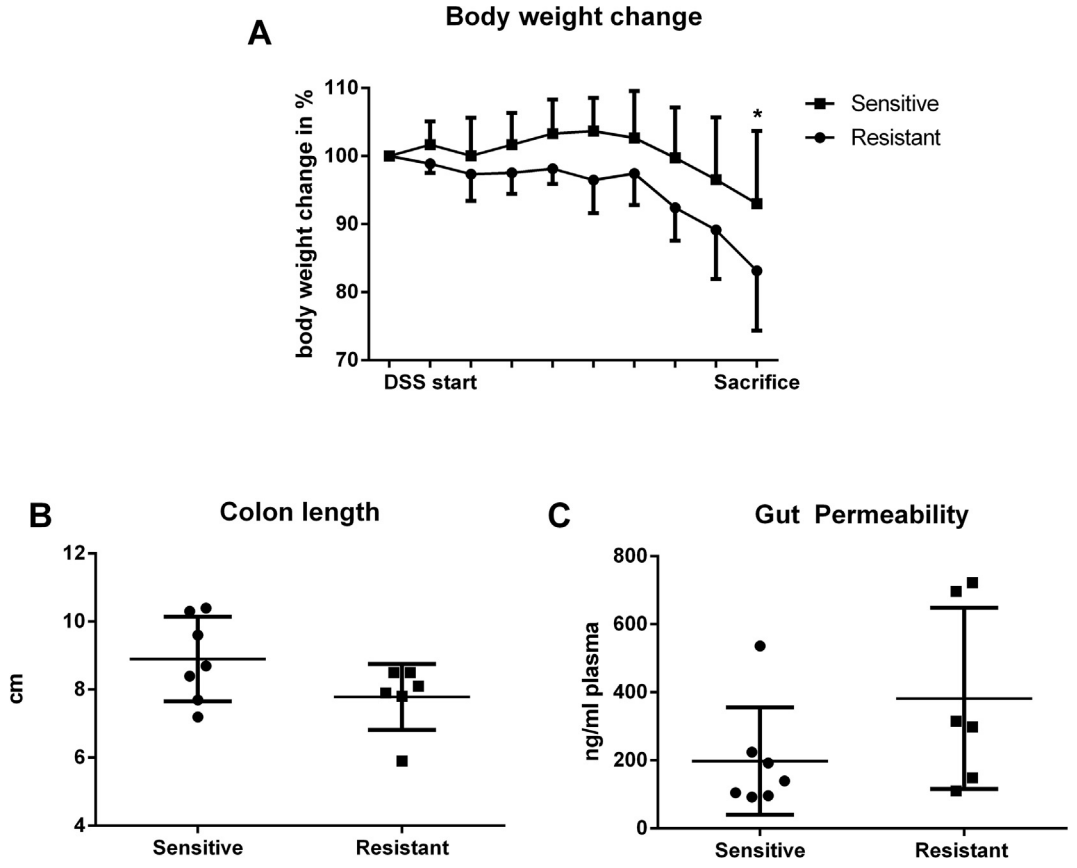

Fig. 2. Colitis severity markers in recipient mice. A: Relative body weight change. Data plotted as mean + SD. $^{*}p < 0,05$. B: Colon length. Mice that received resistant microbiota presented with lower colon lengths. C: Gut permeability. Differences are not significant although trend is showing increased gut permeability in mice that received resistant microbiota.

relatively resistant to the given pathology to healthy donors before the pathology develops. This is called preemptive FMT.

Reverse phenotype transfer, however, means that FMT from the susceptible subject with microbiota that predisposes to the disease to the recipient will lead to increased resistance of the recipient. This could be partially explained by the fact that pretreating healthy subjects with potentially harmful bacteria has immunomodulatory effects that can protect the recipient against the pathology and alleviate its severity.

Evaluation of the hypothesis

Possible Implications of the hypothesis are wide, but indeed depend on its validity. We predict that if the hypothesis of reverse phenotype transfer via preemptive FMT is generally valid, it should be confirmed on other models of inflammatory and possibly also other types of disease. Another possibility is that this phenomenon is only related to gut inflammation (or more specifically, chemically induced inflammation) and thus cannot be applied to other phenotypes. This, however, is less likely as FMT has been previously shown to be a strong tool for transfer of phenotype and therapy, respectively, in several distinct conditions, including obesity, neurological or cardiovascular disorders [14–16]. None of the published studies reported the reverse phenotype transfer, so we assume that this approach has never been studied and described.

In case the hypothesis of reverse phenotype transfer via preemptive FMT is not specific for gut inflammation, it opens brand new avenues for preventive medicine. To test this idea, it is necessary to perform additional experiments on laboratory animals in which the healthy fecal transplant from high susceptibility donors should increase the resistance to the disease in healthy recipients.

To validate the hypothesis, it is necessary to show that the healthy microbiota (or at least some part of it) of the donor is clearly different from the healthy microbiota of the recipient or that the FMT produced structural changes in recipient's microbiome. On top of that it is vital to elucidate the mechanism by which the reverse phenotype transfer affects the hosts. We hypothesize that pretreatment with potentially harmful bacteria may prime the immune system to protect against another hit with same or similar microbiota. An effect similar to vaccination. For this purpose, it is necessary to perform whole array of analyses of the microbiome and the immune system of both donor and recipient, including immune cells flow cytometry, structural and functional microbiota analysis etc. Furthermore, it is crucial to discover how long such an effect lasts and whether it is associated with the transplanted microbiota.

Empirical data

In the pilot experiment the feces from healthy donor mice were collected and frozen for further use. Afterwards, colitis was induced in these donors and based on clinical markers severity of colitis was assessed. Afterwards mice that had less severe colitis were termed as “resistant” and their healthy microbiome was transferred to healthy host. Mice with more prominent colitis were called “sensitive” and their healthy microbiome was also transferred to healthy host. In both hosts, the “resistant” and “sensitive”, colitis was induced after the transplantation. The experimental scheme and anticipated results are shown on Fig. 1a. On the other hand, Fig. 1b shows the observed findings, which represent the proposed hypothesis.

Fecal microbiota transplantation was performed via oral gavage with volume of 0,15–0,2 ml and for 3 or 5 consecutive days, one gavage per day, with the last gavage at the day of DSS intake. Colitis was

induced with dextran sulfate sodium (DSS) in drinking water ad libitum for 7 days [17]. At the 7th day mice were sacrificed, and blood and colon was collected. All organs were weighted, and the length of colon was measured. The experiments were approved by the ethical committee of the Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Slovakia and comply with the animal welfare and ethical guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Two independent experiments were performed that included 4 mice per group. In the second experiment, there was statistical difference between sensitive and resistant hosts in relative body weight loss on the last day of the experiment (Fig. 2A). Mice that were assumed to have worse colitis (sensitive hosts) showed lower weight loss compared to the resistant host that lost more weight. Comparable results were observed measuring colon length (Fig. 2B) and gut permeability (Fig. 2C). These results were unexpected and show findings that are opposite to the anticipated ones.

Consequences of the hypothesis and discussion

Gut microbiota dysbiosis is implicated in pathogenesis of IBD, however, it is still not clear whether it is a cause or effect. Modulation of gut microbiome is a feasible way to explore the contribution of resident microbiome on the severity of experimental colitis. Gut microbiome modulation in DSS colitis using antibiotic pre-treatment has shown controversial results. A study has shown that broad spectrum antibiotic pre-treatment aggravates DSS colitis [18]. Another study showed that broad spectrum and selective gram-positive antibiotics ameliorate DSS colitis, concluding that gram positive bacteria are necessary to mobilize macrophages [19]. In some of our preliminary and published experiments, antibiotics seemed to worsen DSS colitis in CD1 mice strain and seemed to ameliorate DSS colitis in C57BL6 strain, underlining the complexity of host-microbiome interactions [20].

To our knowledge there is no study published to date concerning modulation of microbiome using FMT prior to colitis induction. In our experiments, interventions aimed at modulating the microbiome using FMT modified the severity of colitis in some parameters. Interestingly, the group that received microbiome from mice with the most severe colitis showed less body weight loss and reduced gut permeability than the group that received microbiome from mice with the least severe colitis. Possible explanation could be that pretreating with potentially harmful bacteria has immunomodulatory effects in the long run. Commensal bacteria limit the growth of potential pathogens, however after antibiotic reduction of commensals (as was in our case) pathogens that were either present or introduced with FMT are able to thrive and possibly cross intestinal barrier to end in host's lymphatic system, thus preconditioning the immune system and ameliorating the disease [21]. Moreover, experimental study showed that pretreating mice with either extracellular DNA isolated from mice suffering from colitis or with fecal bacterial lysates attenuated DSS colitis [22]. Another explanation could be that microbial components exert anti-inflammatory response from the host dependent on TLR9 signal pathway as has been previously shown [23].

Relatively high interindividual variability has been observed. For example, two out of seven mice in sensitive group did not respond to the microbiome modulation. Similarly, two out of six mice in resistant group showed lesser weight loss than the other mice from the same group. This is in accordance with recent evidence on individual variability in FMT response. Study from 2015 reported that patients receiving FMT from a donor “B” showed higher rate of response than the others [24]. A different group observed similar event and investigated it further. They found microbial signatures that could predict the efficacy of FMT. Recipients that had low abundancies of Clostridium clusters IV and XIVa while having high abundancies of proteobacteria and Bacteroidetes were facing a poor outcome of FMT. Similarly, donors with high levels of the same Clostridium clusters and low levels of *Ruminococcus gnavus* were more prone to exert beneficial effect [25]. Although the results are still correlative, this is the only study finding bacterial markers of efficacy concerning FMT.

It is difficult to reliably conclude whether the hypothesis was sufficiently supported by observations, because microbiota composition was not analyzed. On top of that it is unclear whether the intervention was a result of FMT alone or in combination with some effects of antibiotics administration.

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Conflict of interest statement

The authors declare no conflict of interest.

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