



Fecal Microbiota Transplantation Enhances Phagocytic Activity and T-cells Immunity Mediated by Bacterial Vaccines

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ABSTRACT

Faecal microbiota transplantation is a next-generation medical therapy for several clinical issues. However, the effect of this approach has not been studied in the context of vaccination. Here, we aimed to use Faecal microbiota transplantation as an adjuvant to enhance both phagocytic activity and T-cell immunity in response to bacterial vaccination. 20 albino mice (5 groups, 4 mice per group) were used in this study. Two groups were subjected to faecal microbiota transplantation with 100 μ l of faecal slurry from a healthy adult via two routes (with or without gut microbiota modulation by a cocktail of antibiotics), then immunised with 100 μ g of a bacterial vaccine. The remaining groups were the control group treated with PBS, the bacterial vaccine-alone immunised group, and the PLGA-encapsulated bacterial vaccine immunised group. The results clearly show that the phagocytic activity of macrophages increased significantly to (74.67 ± 6.11) % in vaccinated mice after Faecal microbiota transplantation with modulation of gut microbiota group compared to both the control group (38.67 ± 2.31) % and vaccinated mice after faecal microbiota transplantation without modulation of gut microbiota (57.33 ± 6.11) .

Also, PLGA-encapsulated bacterial vaccine increases phagocytic activity by $62.67 \pm 8.33\%$. Only vaccinated mice that underwent faecal microbiota transplantation and had gut microbiota modulation showed a significant increase in delayed-type hypersensitivity, with 2.5 ± 0.71 mm compared to 0.25 ± 0.35 mm in the control group.

Overall, enhanced cellular immunity in response to the bacterial vaccine after faecal microbiota transplantation, with modulation of the gut microbiota, suggests that this approach is a promising next-generation bacterial adjuvant.

Keywords: Bacterial vaccines, Faecal microbiota transplantation, Phagocytic activity, T-cell immunity.

INTRODUCTION

A recent medical approach to the treatment of several intestinal diseases is faecal microbiota transplantation (FMT), also known as faecal transplantation, faecal bacteriotherapy, and human intestinal microbiota transfer. FMT includes transferring faeces from healthy persons to ill patients to restore disorders occurring in intestinal microbiota (Ghani *et al.*, 2024a), and was initially considered a promising therapy in the treatment of recurrent *Clostridium difficile* infection (CDI) since 2013 (vanNood *et al.*, 2013), followed by several studies for the same purpose (Ghani *et al.*, 2024a; Mattila *et al.*, 2012). Later, this approach was used in the USA as first-line therapy for CDI (Kelly *et al.*, 2021; McDonald *et al.*, 2018; Peery *et al.*, 2024) and also in Europe (vanPrehn *et al.*, 2021). A history of FMT began in the fourth century in China (reviewed by (Ghani *et al.*, 2024), but the first confirmed report of using FMT as a medical therapy was in the USA in 1958 (Eiseman *et al.*, 1958). The FMT term was first introduced by Bakken *et al.* (Bakken *et al.*, 2011). This method, characterized by several important benefits, is inexpensive, effective, safe, and first-line in the treatment of CDI (Quera *et al.*, 2024). The workflow of FMT includes collecting, mixing, and filtering Faeces from healthy donors, which are then transferred to ill patients via endoscopy or as a capsule (Wang *et al.*, 2019). Adjuvants can be molecules or macromolecules that aim to improve innate immune responses and, when combined with antigens, can significantly modulate and enhance the body's immune response (Jin *et al.*, 2019). Without adjuvants, effective vaccination needs high concentrations and doses of antigens to stimulate effective and long-term immune responses, and this can cause severe damage (Nooraei *et al.*, 2023), so using appropriate adjuvants can significantly reduce these issues that act either as immunomodulant molecules or as delivery systems (Cappellano *et al.*, 2021). Several criteria should be met when using appropriate adjuvants: they should have a safe and effective formulation, induce a long-term and strong immune response across all ages, not stimulate inappropriate or harmful immune responses, and be metabolised in the body (Nooraei *et al.*, 2023). Among them, several adjuvants were used in different types of vaccines. Freund adjuvants depend clearly on the oil-in-water or killed Mycobacterium and have been previously used in various vaccine formulations. In contrast, mineral adjuvants like Alum have been extensively used in both viral vaccines, such as HPV, HBV, tetanus, meningococcal, and Corona vaccines (Oberg *et al.*, 2011), and other types of adjuvants were extensively used, such as complement components (Prater *et al.*, 2006), cytokines, and bacterial-derived adjuvants (Spickler and Roth, 2003). Despite the huge use of these adjuvants, many adverse side effects such as fever, liver necrosis, systemic inflammation, and granulomas, were clearly observed in many types of adjuvants (Debess *et al.*, 2010), as well as other types identified as neurological toxins and can stimulate severe neurological disorders (Bellavite, 2020), so screening for new types of safe and effective adjuvants is necessary.

Therefore, the present study was designed to evaluate the immunomodulatory potential of faecal microbiota transplantation (FMT) as a novel, safe, and cost-effective adjuvant to address the safety concerns and side effects associated with traditional vaccine adjuvants. Specifically, we investigated whether FMT could enhance cellular immune responses induced by a model bacterial vaccine, thereby providing insight into its utility as a novel vaccine adjuvant.

MATERIALS AND METHODES

Ethic consideration

Experimental protocols, including albino mice, were reviewed and approved by the Institutional Animal Care and Use Committee/University of Mosul/College of Veterinary Medicine (ref: UM.VET.2025.114 at 15/7/2025).

Vaccine construction

The chimeric uropathogenic *Escherichia coli* vaccine of the present study was previously developed. Furthermore, previously loaded onto PLGA nanoparticles, which are approved by the FDA as a promising novel delivery system, a water-in-oil-in-water (W1/O/W2) double emulsion

solvent evaporation method was used. The protocol described by Unsunnidhal *et al.* (2021) was depended.

Faecal microbiota transplantation and immune challenging

The FMT protocol used in the present work follows the method described by Parker *et al.* (2022). In brief, 100 g of fresh faeces from a healthy adult was mixed and homogenised with 10 ml of sterile PBS. 100µL of faeces slurry was given orally for 3 consecutive days, and after 24 h, both groups were challenged with the developed bacterial vaccine.

Immune challenging

Twenty albino mice (6-8 weeks) were divided into 5 groups (4 mice/group), housed in clean separate cages and monitored for a month to exclude any unhealthy mice from the experiment. The first group was treated intraperitoneally with 100µl of PBS; the second and third groups were immunised intraperitoneally with 100 µg of the purified bacterial vaccine and 100µg of PLGA nanoparticle +100µg of the purified bacterial vaccine, respectively. The fourth group received FMT with gut microbiota modulation, then was immunised with 100µg of the purified bacterial vaccine.

The last group also received FMT after mice were treated orally with 100 mg/kg of both broad-spectrum antibiotics, ciprofloxacin and levofloxacin, twice daily to wash out gut microbiota, the mice were intraperitoneally injected with 100µl of 100µg MEV three times at 7, 14, and 30 days.

Phagocytosis assay

Isolation of peritoneal macrophages

A method described by Ray and Dittel (2010) was used to isolate peritoneal macrophages. In brief, 2 mice from each group were euthanised using a mixture of ketamine and xylazine, 5 ml of PBS was injected intraperitoneally, the peritoneal cavity was gently massaged and opened with a scalpel, and all peritoneal fluids were pipetted into 1.5 ml Eppendorf tubes.

Measurement of phagocytic assay

Phagocytic activity of macrophages assayed using a method of Gonçalves and Mosser (2015) with some modifications: Isolated peritoneal fluids centrifuged at 6000 rpm for 5 min. Supernatant removed, and pellets resuspended with 1 ml of PBS. 0.1 ml of resuspended peritoneal fluid was smeared on a glass slide in duplicate and incubated at 37°C in a 5% CO₂ atmosphere for 1 hour. After 1 hour of incubation, all slides were washed thoroughly using PBS. 0.1 of *E. coli* (used in bladder challenging) bacterial suspension was spread on the slides, and re-incubated. Then re-washed. Phagocytic activity was counted using the following formula:

$$\text{Phagocytic activity (\%)} = \frac{\text{Number of engulfed macrophages}}{\text{Total number of macrophages}} \times 100$$

Measuring T-cell immune response

Delayed-type hypersensitivity (DTH) reveals T-cell-mediated immunity to bacterial vaccines. The test was performed according to Bernal *et al.* (2023). 5 µg of the bacterial vaccine from all formulations, in 0.04 ml, was injected into the left footpad of the mice; the same volume of normal saline was subsequently injected into the right footpad. DTH was evaluated through the thickness of the footpads as follows:

$$\Delta \text{mm swelling} = \text{mm of left footpad} - \text{mm of right footpad}$$

Visualisation and statistical analysis

GraphPad Prism 8 was used for statistical analysis and data visualisation in the present study, all parametric results are visualised as Mean ± Standard deviation, followed by a One-way ANOVA at P = 0.05, and the Tukey multiple-comparison test was used to compare the concentrations of Cellular immunological parameters (DTH and phagocytic activity).

RESULTS AND DISCUSSION

The present work assesses the candidate MEV's capability to activate cellular immunity by evaluating its role in stimulating both DTH and phagocytic activity. The statistical analysis, as shown in (Table 1) and Fig. (1) indicates that the candidate MEV induce DTH non-significantly when used alone, encapsulated form, and after FMT without gut modulation, whereas this increase where clearly more and significantly ($P<0.05$) after FMT with gut modulation, as footpad thickness increased to (2.5 ± 0.71) mm compared to (0.25 ± 0.35) mm in the control group.

Table (1): Effectiveness of bacterial vaccine in enhancing phagocytic activity and DTH with different formulations.

Cellular immunity	Control	MEV	PLGA	FMT	A/F
	Mean $\pm$ SD				
DTH (mm)	0.25 $\pm$ 0.35	1.25 $\pm$ 0.35	1.25 $\pm$ 0.35	1.25 $\pm$ 0.35	2.5 $\pm$ 0.71
Phagocytic activity (%)	38.67 $\pm$ 2.31	54.67 $\pm$ 10.07	62.67 $\pm$ 8.33	57.33 $\pm$ 6.11	74.67 $\pm$ 6.11

MEV = injection of purified 100 μ g of the bacterial vaccine, PLGA = injection of purified 100 μ g of the PLGA-encapsulated bacterial vaccine, FMT = immunisation with 100 μ g of the bacterial vaccine after FMT, A/F= immunisation with 100 μ g of the bacterial vaccine after washing gut microbiota and FMT.

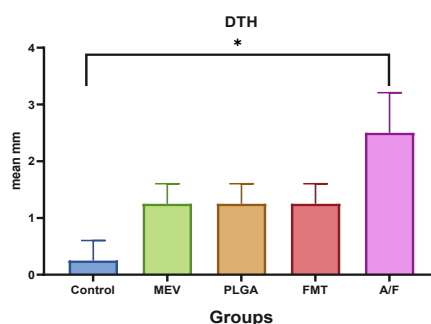


Fig. 1: Stimulating activity of the candidate MEV on DTH. MEV= injection of purified 100 μ g of the bacterial vaccine, PLGA = injection of purified 100 μ g of the PLGA-encapsulated bacterial vaccine, FMT = immunisation with 100 μ g of the bacterial vaccine after FMT, A/F= immunisation with 100 μ g of the bacterial vaccine after washing gut microbiota and FMT.

Fig. (2) show that only two MEV formulation cause a significant ($P<0.05$) increase in the phagocytic activity of the isolated peritoneal macrophages, PLGA-encapsulated MEV rises the activity to (62.67 ± 8.33) % compared to (38.67 ± 2.31) % in the control group, while immunisation after FMT with gut modulation cause the highest increases in the phagocytic activity of macrophages, which amount to (74.67 ± 6.11) %. Fig. (3) shows the different stages of the phagocytic activity assay.

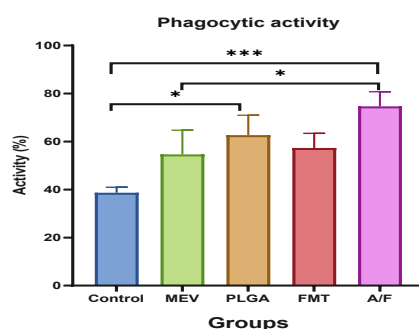


Fig. 2: Stimulating activity of FMT on phagocytic activity. MEV = injection of purified 100 μ g of the bacterial vaccine, PLGA = injection of purified 100 μ g of the PLGA-encapsulated bacterial vaccine, FMT = immunisation with 100 μ g of the bacterial vaccine after FMT, A/F= immunisation with 100 μ g of the bacterial vaccine after washing gut microbiota and FMT.

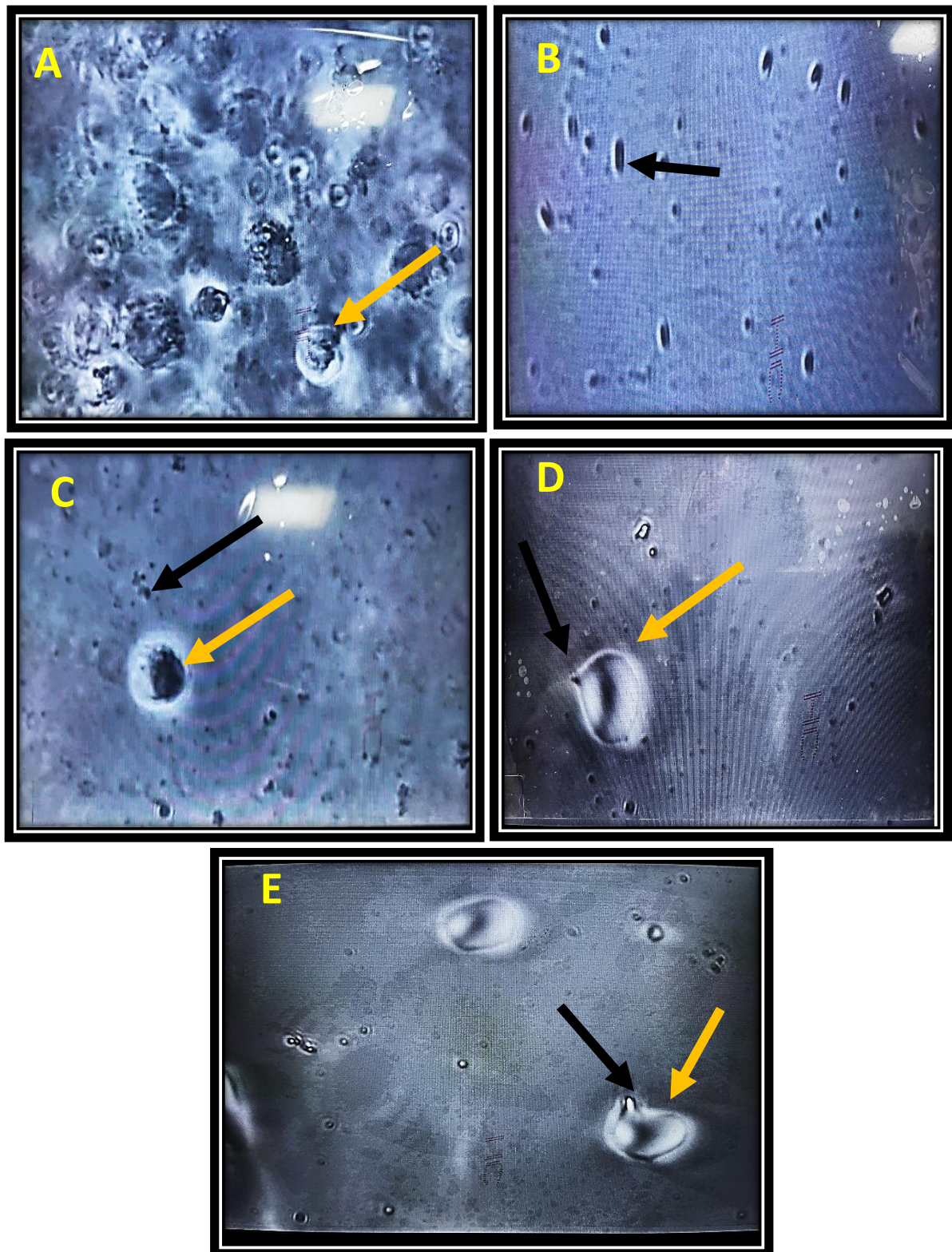


Fig. 3: Phagocytosis by isolated macrophages. Crude cells of peritoneal fluid (A). bacteria (B). isolated macrophage with bacteria (C). Positive bacterial engulfed macrophage (D). Positive and negative engulfed macrophages (E). (Yellow arrows refer to macrophages, black arrows refer to bacteria).

In the present study, we assess the effect of FMT on the immunogenicity of the bacterial vaccine, which triggers cellular immune responses. Only immunisation after FMT with gut modulation triggers a significant increase in macrophage phagocytic activity, consistent with our hypothesis that FMT may promote phagocytic activity, thereby enhancing presentation of vaccine antigenic determinants. On the other hand, PLGA-encapsulated MEV enhances only phagocytosis activity.

Phagocytosis is one of the most important innate immune responses that mediate the engulfment of microorganisms, such as bacteria, and trigger other immunological pathways (Koro and Sharif, 2022). On the other hand, DTH is defined by a beneficial immunological reaction mediated by both T cells and cytokines, which in turn cause secretion of many types of cytokines that affect and regulate other immunological reactions (Kobayashi *et al.*, 2001), and this clearly suggests inducing balanced humoral and cellular immune responses by the bacterial vaccine of the present work by the action of the FMT. So, increased phagocytic activity of macrophages may be correlated with increased INF- γ levels, as it has been well noted that INF- γ significantly improved phagocytosis (Imran *et al.*, 2025; Lake *et al.*, 2025). Also, the increased DTH observed in the present study may be linked to increased INF- γ (Peyer *et al.*, 2025).

The exact underlying mechanism by which FMT enhances the immunogenicity of a bacterial vaccine is not fully addressed in the present work. However, it may be due to altering the gut microbiota to enhance the predominance of certain bacterial species, known as probiotics. As the study of Paramsothy *et al.*, (2019) suggests, the abundance of some types of bacterial species, such as a probiotic *Bacteroides* spp related to positive FMT outcomes in cases of ulcerative colitis, as well as this bacterial species, consequently increases production of short-chain fatty acids (SCFA), while other types of bacteria, like *Streptococcus* spp, are responsible for negative outcomes. It concludes that SCFA types, such as butyrate, play a crucial role in maintaining gut health and regulating immune responses. Later, studies clearly demonstrate that the transfer of probiotics via FMT is significantly associated with beneficial immune responses (Amoroso *et al.*, 2020; Frisbee and Petri, 2020; Zeng *et al.*, 2019; Zhang *et al.*, 2020), including *Bacteroides* spp. Other probiotic bacteria, such as *Akkermansia muciniphila*, play an important role in tissue repair by activating anti-inflammatory cytokines, such as IL-10, and by producing SCFA (Zhang *et al.*, 2019). In conclusion, FMT has also been used to treat other medical conditions such as constipation (Cao *et al.*, 2017) and colorectal cancer (Sui *et al.*, 2020; Zhang *et al.*, 2017). These effects highlight the immune-modulatory effect of FMT and suggest probable adjuvant effects.

Despite the beneficial immunomodulatory effect of the FMT in the present work, this study has a limitation: The exact modulation of the gut microbiota has not been studied, so it needs to conduct other similar future studies in line with conducting metagenomic sequencing to correlate the abundance of bacterial species with the positive outcomes of the FMT.

CONCLUSION

This study highlights the importance of manipulating gut microbiota to direct and enhance the immunogenicity of bacterial vaccines. The experimental results showed that faecal microbiota transplantation (FMT) has strong adjuvanticity and can significantly enhance cell-mediated immunity by increasing the phagocytic activity of peritoneal macrophages and promoting T-cell-mediated delayed-type hypersensitivity (DTH) responses. Importantly, the results highlight that preconditioning of the recipient's gut microbiota with broad-spectrum antibiotic cocktails is a prerequisite for optimal immunomodulatory effects. Immunisation after FMT and gut modulation elicited a statistically superior immune response compared with FMT protocols without prior microbial depletion.

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تعديل المحتوى الميكروبي المعوي عبر النقل البرازي: آفاق جديدة لتعزيز المناعة الخلوية للقاحات البكتيرية

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الملخص

يعد عملية نقل المحتوى الميكروبي البرازي جيل جديد وواعد لعلاج العديد من المشاكل الطبية. وبالرغم من ذلك، فعالية هذا النهج في عملية التحصين لم تُدرس لحد الآن. لذا هدفت الدراسة الحالية لاستخدام نقل المحتوى الميكروبي البرازي كمساعد مناعي لتحسين الاستجابة المناعية الخلوية (الفعالية البلعمية للبلاعم الكبيرة واستجابة الخلايا التائية) للقاحات البكتيرية. تم استخدام 20 من الفئران البيض (5 مجاميع، 4 فئران لكل مجموعة). خُضعت مجموعتين من الفئران لعملية نقل المحتوى الميكروبي البرازي بـ 100 مايكرو لتر من مزيج برازي من شخص بالغ سليم وبطريقتين (مع أو بدون تعديل المحتوى الميكروبي المعوي للفئران بواسطة خليط من المضادات الحيوية)، ثم مُنعت بـ 100 مايكرو غرام من لقاح بكتيري. عوملت المجاميع المتبقية بالمحلول الداري كمجموعة سيطرة، لقاح بكتيري لوحده، لقاح بكتيري مُحمل على الدقائق النانوية PLGA.

اظهرت النتائج بشكل واضح أن الفعالية البلعمية للبلاعم الكبيرة ارتفعت معنوياً الى (74.67 ± 6.11) % في الفئران الممنعة بعد نقل المحتوى الميكروبي المصاحب بعملية تعديل المحتوى الميكروبي للأمعاء مقارنة بمجموعة السيطرة (38.67 ± 2.31) %، وكذلك مقارنة بالفئران الممنعة بعد نقل المحتوى الميكروبي من دون التعديل (57.33 ± 6.11) %. وبينت النتائج ارتفاع استجابة الخلايا التائية فقط لدى الفئران الممنعة بعد نقل المحتوى الميكروبي المصاحب بعملية تعديل المحتوى الميكروبي للأمعاء مقارنة بمجموعة السيطرة. بشكل عام، ان تحسين الاستجابة المناعية الخلوية للقاح البكتيري بعد عملية نقل المحتوى الميكروبي المصاحب بعملية تعديل المحتوى الميكروبي للأمعاء يشير بشكل واضح بأن هذا النهج يعد جيل جديد واعد من المساعدات المناعية.

الكلمات الدالة: اللقاحات البكتيرية، نقل المحتوى الميكروبي البرازي، الفعالية البلعمية، استجابة الخلايا التائية.