Author/s:
Loke, P;Heine, RG;McWilliam, V;Cameron, DJS;Tang, MLK;Allen, KJ

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# Fecal microbial transplantation in a pediatric case of recurrent *Clostridium difficile* infection and specific antibody deficiency

Authors: Paxton Loke, MBBS [a], Ralf G Heine, MD, FRACP [a,b,c,d], Vicki McWilliam BSc, MND [a,d], Donald JS Cameron, MBBS, FRACP [b,c,d], Mimi LK Tang MBBS, FRACP, FRCPA, FAAAAI, PhD [a,c,d], and Katrina J Allen, MBBS, BMedSc, FRACP, FAAAAI, FAAHMS, PhD [a,b,c,d,e]

Affiliations:

From [a] the Department of Allergy and Immunology, Royal Children's Hospital, Parkville, Victoria, Australia; [b] the Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Parkville, Victoria, Australia; [c] the Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia; [d] the Murdoch Childrens Research Institute, Parkville, Victoria, Australia, [e] Institute of Inflammation and Repair, University of Manchester, UK.

Corresponding author: Professor Katrina J. Allen, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Rd, Parkville 3052, Victoria, Australia. Tel.: +613 99366752. Fax: +613 9345 4848. Email: katie.allen@rch.org.au.

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To the Editor:

Fecal microbial transplantation (FMT) has been shown to be more effective than antimicrobial therapy in the treatment of recurrent *Clostridium difficile* infection (CDI) in adults, with a randomized control trial (RCT) showing resolution of symptoms and clearance of CDI in 81% of subjects compared to 31% with standard antibiotic therapy (1) . In a systematic review of all studies evaluating FMT for treatment of CDI to date, including 2 RCTs and 21 case series (involving more than 500 patients), it was concluded that FMT is a promising treatment for recurrent CDI in adults with relatively good short-term safety data (2). Reports have so far mainly remained in the realm of adults, and data on FMT in young children are limited. This is an important gap since the first 2-3 years of life is associated with the establishment of a normal gut microbiome pattern as well as establishment of oral tolerance. We report a unique case of a FMT in a 2 year old boy who had recurrent CDI resistant to standard antimicrobial treatment and a diagnosis of specific antibody deficiency (SAD).

A 14-month-old boy was referred to our tertiary center for investigation of recurrent diarrhea, presumed non-IgE-mediated allergy to cow's milk and soy and recurrent infections. The patient was born by normal vaginal delivery and was breastfed until 4 months of age with mother on an unrestricted diet. Solids had been introduced at 6 months and at the time of presentation included fruit, vegetables, rice and some cow's milk products (cheese and yoghurt) but no formula. Reported multiple infections in the first 12 months of life included rotavirus gastroenteritis at 8 months of age, recurrent otitis media which required multiple courses of oral antibiotics and recurrent fevers which were presumably viral upper respiratory tract infections (but were treated with oral antibiotics on most occasions between 6 and 14 months). Gastroscopy and colonoscopy were normal and found no gastrointestinal cause for his persistent diarrhea, in particular no evidence of celiac disease or colitis. Initial immune investigations revealed normal serum IgG, IgA and IgM, and normal specific antibody responses to protein antigens. Antibody responses to polysaccharide antigens were not assessed, as interpretation of these is difficult in infancy.

The patient was commenced on an amino-acid formula (AAF) and cow's milk and soy elimination with some improvement in irritability and decrease in stool output although loose bowel actions continued. Reintroduction of cow's milk and soy was attempted on several occasions but was followed by significant worsening of his diarrhea so he remained on AAF while strictly avoiding CM and soy. Despite this, he continued to thrive with height and

weight percentiles consistently being above the 90$^{th}$ percentiles (WHO charts). At age 18 months, *Clostridium difficile* toxin (CDT) was detected in feces which was initially treated with a course of metronidazole with resolution of his diarrhea for several weeks.

From the age of 18 months until 23 months of age, he continued to suffer from recurrent episodes of diarrhea with up to 5 to 6 loose stools per day. CDT was again detected in his stools at 20 months, 21 months and 26 months of age, respectively. This was despite repeat antibiotic treatment with courses of metronidazole, followed by oral vancomycin on separate occasions. After a prolonged course of oral vancomycin, the diarrhea and CDT relapsed on weaning (before complete withdrawal) of the antibiotic. In the interim, he had further infections including adenovirus (proven by PCR) and infestation with *Blastocystis hominis* (found on stool microscopy) at age 23 and 26 months, respectively and an episode of *Staphylococcus aureus* bacteremia (age 27 months). Due to his recurrent antibiotic resistant *C difficile* infection, FMT was considered at this stage, with his father as a possible donor.

Given the child's history of continuing recurrent infections, more extensive immunological workup was conducted prior to FMT. The patient was fully immunized and received the conjugated pneumococcal vaccine Prevenar-13 at the age of 2, 4 and 6 months under the Australian National Immunization Program Schedule. He was then immunized with Pneumovax-23 at the age of 29 months. These revealed a diagnosis of likely specific antibody deficiency (SAD) with reduced specific antibody responses to polysaccharide antigens in the presence of normal total IgG (3, 4). Evaluation of pre and post Pneumovax-23 immunization serotype-specific IgG titres showed that the patient had poor functional antibody response to Pneumovax-23, with adequate responses to only 3 of the 8 polysaccharide serotypes contained within Pneumovax-23 (and not the conjugated Prevenar-13 vaccine), where an adequate response is defined as a post vaccination IgG titre of ≥ 1.3mcg/mL or ≥ 4 fold rise in titre from pre immunization for ≥ 50% of serotypes tested (5, 6). Responses to conjugated serotypes within Prevenar-13 were normal (adequate response to 7 of 7 serotypes). These results are shown in Table 1. As one of the possible immunological manifestations of interleukin-1-receptor associated-kinase-4 (IRAK4) deficiency is specific antibody deficiency, the patient was investigated and had normal CD62L shedding on granulocytes, thus excluding IRAK4 deficiency (7). Other immune investigations were normal (investigations summarized in Table 2). IgG subclasses were not measured in this study. The clinical significance of IgG subclass deficiency is controversial and functional antibody responses are generally considered to be of greater relevance in determining the likelihood of susceptibility to recurrent infections (8, 9). Aside from intermittent courses of

antibiotics, this patient did not receive any intravenous immunoglobulin treatment. Further genetic investigations were not undertaken as monogenic causes for SAD are yet to be identified (10).

Given the diagnosis of SAD, the young age, and the limited evidence for FMT in this clinical setting, an application for FMT was submitted to and approved by the Royal Children's Hospital New Technologies' Committee. The donor was the child's father who underwent screening which included stool microscopy and culture, stool virology (rotavirus, adenovirus and enterovirus), CDT and a screen for transmissible diseases including hepatitis A, B, C and HIV. All tests were negative. The donor was also asked to exclude cow's milk from his diet in order to minimize the possibility of allergen exposure given the recipient (his child) had non-IgE-mediated cow's milk allergy. At the age of 30 months, after informed consent by both parents, our patient underwent FMT with a 150 mL suspension (50 g donor stool was suspended in 500 mL of 0.9% sodium chloride solution) infused over 10 minutes into his cecum via colonoscopy. There was a rapid and dramatic improvement in his bowel movements with a formed stool (Bristol stool type 4) occurring as the first output 24 hours post FMT - his first formed stool ever. Repeated CDT testing on subsequent weeks remained negative and the child has remained CDT negative for 18 months post FMT despite at least four courses of oral antibiotics for presumed protracted bacterial bronchitis and otitis media. He also had a second episode of culture-proven bacterial illness (*Staphylococcus aureus* bacteremia at 31 months). He is tolerating all foods including cow's milk with an unrestricted diet at 48 months of age and his stool patterns remain normal.

In summary, FMT from an adult- related donor to a young child was effective in the clearance of recurrent CDI, and did not result in FMT-associated bacterial translocation or sepsis even in the setting of a diagnosis of Specific Antibody Deficiency. This is one of 3 reported cases of FMT in a child less than 3 years and the first reported case in the setting of a primary immune deficiency. Although his cow's milk protein intolerance also appears to have resolved we can only speculate if this occurred in response to the FMT procedure.

Table 1: Evaluation of the patient's polysaccharide antibody response following 5 weeks post immunization with Pneumovax-23.

| Serotypes contained in both Pneumovax-23 and Prevenar-13 | Pre (mcg/ml) | Post (mcg/ml) |
|---|---|---|

| | | |
|---|---|---|
| 4 | <0.1 | >4.1 |
| 6B | <0.1 | >8.5 |
| 9V | <0.1 | 2.0 |
| 14 | 1.0 | >13.9 |
| 18C | <0.1 | >3.4 |
| 19F | 0.2 | >13 |
| 23F | <0.1 | 3.2 |
| Serotypes contained in Pneumovax-23 only (pure polysaccharide antibody response) | | |
| 2 | 0.1 | 7.1 |
| 8 | <0.1 | >5.1 |
| 10A | <0.1 | <0.1 |
| 11A | <0.1 | 0.3 |
| 15B | 0.8 | 0.7 |
| 17F | 0.1 | 0.1 |
| 20 | 0.3 | 1.9 |
| 33F | <0.1 | 0.2 |

**Table 2**: Summary of patient's immunological investigations, prior to FMT. Normal reference ranges in brackets.

| Investigation | Result (age specific normal range) |
|---|---|
| Immunoglobulins | |
| IgG | 8.42 g/L (3.4-19.20) |
| IgA | 0.87 g/L (0.48-3.45) |
| IgM | 0.61 g/L (0.43-1.63) |
| IgE | 8 kU/L (0-35) |
| Vaccine antibodies | |
| Tetanus toxoid IgG | 0.31 IU/mL (>0.2 IU/mL) |
| *Haemophilus influenzae* B IgG | 0.35 mcg/mL (>0.15 mcg/mL) |
| Pneumococcal IgG (5 weeks post Pneumovax-23 vaccination) | Inadequate response (<1.3 mcg/ml or less than four-fold rise in titer following immunization) to 5 of 8 polysaccharide-only serotypes contained within Pneumovax-23. |
| Lymphocyte subsets | |
| CD3+/CD4+ | 2.33 x $10^9$/L (0.5-2.4), 44% (23-48) |
| CD3+/CD8+ | 1.27 x $10^9$/L (0.3-1.6), 24% (14-33) |
| CD19+ | 1.00 x $10^9$/L (0.2-2.1), 19% (14-44) |
| CD16+/CD56+ | 0.26 x $10^9$/L (0.1-1.0), 5% (4-23) |
| Naïve T cells | |
| CD3+/CD4+/CD45RA+/CD62L+ | 2.29 x $10^9$/L (0.42-1.50), 83% (50-85) |
| CD3+/CD8+/CD45RA+/CD62L+ | 1.10 x $10^9$/L (0.26-0.85), 74% (42-81) |
| Memory B cells | |
| CD19+/CD27+ | 0.17 x $10^9$/L (0.05-0.39), 16.9% (10-30) |
| CD19+/CD27+/IgM+/IgD+ | 0.11 x $10^9$/L (0.04-0.21), 10.9% (4.9-14.2) |
| CD19+/CD27+/IgM-/IgD- | 0.05 x $10^9$/L (0.02-0.12), 4.7% (2.9-9.2) |
| Mitogen proliferation | |
| PHA | Normal |
| Anti-CD3 | Normal |

| | |
|---|---|
| Neutrophil oxidative burst | Normal |
| CD62L shedding on granulocytes | Normal |

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