Fecal cytokine profile as markers of intestinal inflammation in *Aotus nancymaae* diarrhea model for enteropathogens

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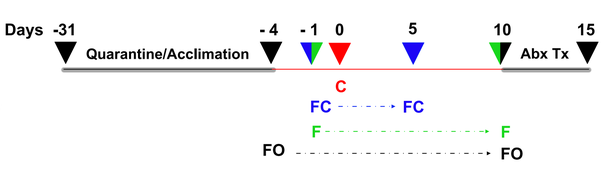
Abstract

U.S. Naval Medical Research Unit No. 6 (NAMRU-6) established an animal model that closely resembles diarrheal diseases caused by *Shigella*, *Campylobacter*, and enterotoxigenic *Escherichia coli* (ETEC) in humans. These enteropathogens cause considerable mortality due to diarrhea in children and morbidity in travelers as well as military personnel deployed to regions of high prevalence. The successful oral challenge model developed in *Aotus nancymaae*, a New World monkey species, for *Shigella*, *Campylobacter*, and ETEC results in reproducible diarrhea attack rates. This model has been fundamental for the development and evaluation of new vaccine candidates, passive therapeutic and prophylactic treatments to prevent infection, inflammation, or diarrhea. However, the immune response and intestinal inflammation by the main enteropathogens are not clearly defined. This study investigates how the cytokines of intestinal inflammation respond to gastroenteritis in *A. nancymaae*. Twenty-nine fecal cytokines (FC) were measured during diarrhea induced by *Shigella flexneri* (N = 8), *Campylobacter jejuni* (N=8), and ETEC (N = 8). Understanding the dynamic relationships between pro-inflammatory and anti-inflammatory cytokines, as well as induction of chemokines and growth factors after the challenge will help us better understand the course of infection by these enteropathogens.

Animal study design



C –challenge; FC –fecal cytokines analysis; F –fecal collection, all animals; FO – fecal observation and scoring, all animals; Abx Tx – Antibiotic treatment.

Demographic variable and study groups

Pathogen	Demographic variable				Study groups			
	N° of animals	N° males/ famles	Mean age, months (SD)	Mean weight, grams (SD)	Strain	Dose CFU	Challenge day	Route
ETEC	12	6/6	16.2 (1.3)	812.5 (72.3)	H10407	$5x10^{11}$	0	OG
Shigella flexneri	12	6/6	14.2 (1.6)	704.2 (56.0)	2457T	$1x10^{11}$	0	OG
Campylobacter jejuni	11[a]	5/6	14.8 (3.0)	757.9 (63.8)	CG8421	$5x10^{12}$	0	OG

[a] One animal excluded from data analysis due to the presence of diarrhea for 3 days prior to challenge.

Attack-rate

Onset and duration of diarrhea after oral challenge of *Aotus nancymaae* with different enteric pathogens.

Challenge strain	No of animals	Diarrhea[a]			
		No of cases	Attack-Rate	Mean no. of days to onset (range)	Mean no. of days to illness (range)
ETEC H10407	12	7	58%	2.3 (1-7)	3.1 (2-6)
S. flexneri 2457T	12	6	50%	5.5 (4-6)	3.6 (2-5)
C. jejuni CG8421	11	8	73%	3.5 (1-6)	2.8 (2-4)

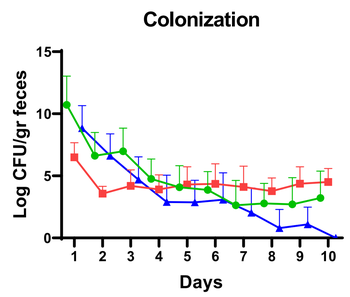
[a] Diarrhea defined as at least one lose-watery stool on at least two consecutive days during the observation period (10 days) post challenge.

Colonization

Onset and duration of colonization after oral challenge of *Aotus nancymaae* with different enteric pathogens.

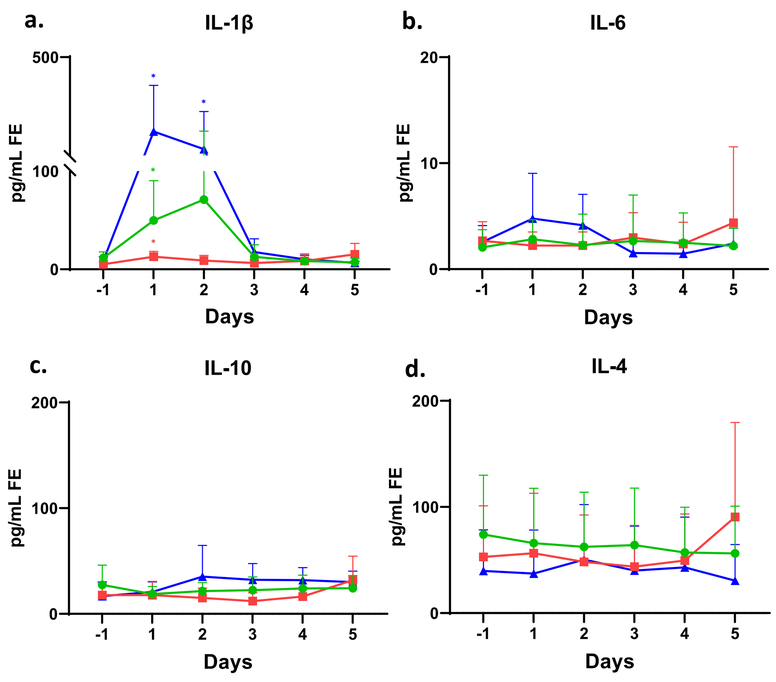
Challenge strain	Fecal Shedding[a]		
	Incidence (%)	Onset	Days of duration (range)
ETEC H10407	100	1	8.8 (5-10)
S. flexneri 2457T	100	1	7.4 (3-9)
C. jejuni CG8421	100	1	9.6 (7-10)

[a] Fecal shedding assessed by plating on solid agar with confirmatory colony blotting.



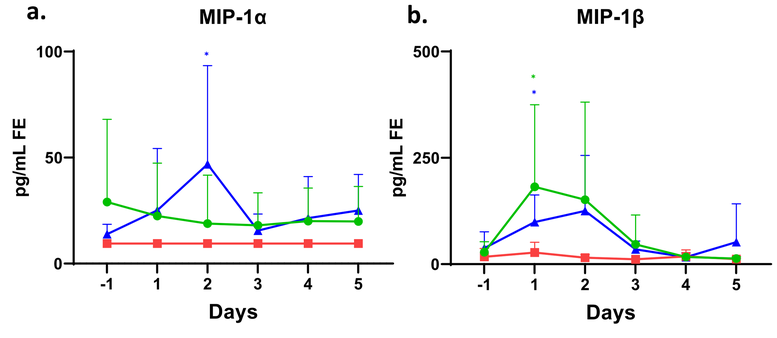
The CFU of ETEC (●), *Shigella flexneri* (▲) and *Campylobacter jejuni* (■) shed in stool from the Day 1 post-challenge until the subjects were treated with enrofloxacin (Day 10).

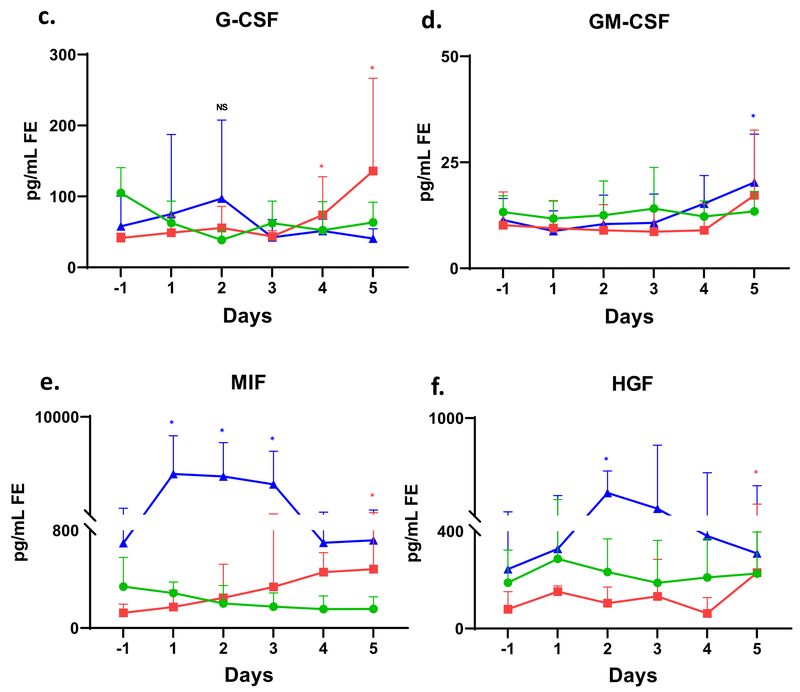
Pro- and anti-inflammatory fecal cytokines



Kinetics of fecal IL-1β (a), IL-6 (b), IL-10 (c) and IL-4 (d). Interleukin concentrations pre- and post ETEC (●), *Shigella flexneri* (▲) and *Campylobacter jejuni* (■) challenge. The *p* values were calculated using the T-test with significance defined as $p < 0.05$.

Fecal chemokines and growth factors profile





Kinetics of fecal MIP-1a (a), MIP-1b (b), G-CSF (c), GM-CSF (d), MIF (e) and HGF (f). Interleukin concentrations pre- and post ETEC (●), *Shigella flexneri* (▲) and *Campylobacter jejuni* (■) challenge. The *p* values were calculated using the T-test with significance defined as $p < 0.05$.

Conclusions

After ETEC and *S. flexneri* infection, the pro-inflammatory cytokines IL-1β increased exponentially, in contrast to the anti-inflammatory cytokines IL-10 and IL-4. However, after infection with *C. jejuni*, intestinal colonization did not fully correlate with FC profiles, with only a slight but statistically significant increase in the pro-inflammatory cytokine IL-β observed on Day 1.

The FC profiles display significant differences between ETEC against *S. flexneri* and *C. jejuni* in the induction of chemoattractant chemokines such as MIF, MIP-1α, G-CSF and GM-CSF as well as in the severity of the inflammatory response (HGF). HGF is a key cytokine during periods of acute inflammation, modulating epithelial cell restitution within the lamina propria.

This information, together with other data such as the onset and duration of diarrhea, will aid in identification of optimal timepoint(s) for the administration of new therapeutic treatments.

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Human subject research protections: Does not apply