Effect of Dietary Monosaccharides on *Pseudomonas aeruginosa* Virulence

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Abstract

Background: *Pseudomonas aeruginosa* is an opportunistic, gram-negative pathogen associated with many hospital-acquired infections and disease states. In particular, *P. aeruginosa* has been identified as a crucial factor in the pathogenesis of neonatal necrotizing enterocolitis (NEC). This condition presents more frequently in infants fed a formula-based diet, which may be a result of the specific monosaccharide content of this diet. We hypothesized that *P. aeruginosa* would express virulence genes differentially when exposed to monosaccharides present in formula versus those in human milk.

Methods: Using the results of a metabolomics study on infant diets and their resulting fecal samples, we identified several monosaccharides that distinguished milk from formula diets. Of these compounds, four were found to be metabolized by *P. aeruginosa*. We subsequently grew *P. aeruginosa* in tryptic soy broth (TSB) supplemented with these four monosaccharides and used quantitative reverse transcriptase-polymerase chain reaction to measure the expression of 59 major *P. aeruginosa* virulence genes. The results were standardized to an external control of *P. aeruginosa* grown in TSB alone.

Results: *P. aeruginosa* did not respond differentially to the monosaccharides after 6 h of growth. However, after 24 h, the organism grown in arabinose (present in formula), xylose (present in human milk), and galactose (present in both formula and feces from milk-fed infants) displayed a significant increase in the expression of virulence genes in all categories. In contrast, *P. aeruginosa* grown in mannose (present in the feces of milk-fed infants) displayed a significant decrease in virulence gene expression.

Conclusion: These results demonstrate the importance of nutrient content on the relative expression of virulence genes in pathogens that colonize commonly the gut of infants. Understanding the effect of current dietary formulas on virulence gene expression in various gut-colonizing pathogens may present a new approach to elucidating the differences between human milk and formula in the development of NEC.

THE INFANT GASTROINTESTINAL TRACT, sterile at birth, undergoes a rapid yet variable colonization by microorganisms during the first year of life. Initially, this colonization is dominated by maternally derived microbes, with the mode of delivery having an evident, although ill-defined, role in the process [1–4]. However, as time progresses, a combination of host genetics and environmental factors quickly adds variability to the infant's intestinal microbiome, ultimately producing a "bioreactor" that, when free of acute processes such as disease and drug therapy, will remain relatively stable throughout life, with only minor changes attributable to aging [3,4].

Of the environmental factors that select the early population of intestinal microbes in a given "bioreactor," the infant

diet has received special attention. By shaping the chemical composition in the gut, many diets establish a microbiome rich in commensal organisms that are beneficial to the host. This process has been illustrated clearly in numerous reports showing that the galacto-oligosaccharides (GOS) of prebiotics and human milk select for a predominance of bifidobacteria [5–8]. In turn, these commensal organisms assist the host with vital functions such as nutrient processing, immune system stimulation, and pathogen defense [5,6,8,9]. Nevertheless, other diets, selecting for a different population of intestinal microbes via the same mechanism, may disrupt the normal balance of commensals and generate the potential for inflammation and disease, as seen in inflammatory bowel disease (IBD), obesity, and neonatal necrotizing enterocolitis (NEC) [1,6,8–10].

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Although many studies have investigated the complex relations among diet, the intestinal microbiome, and disease, few have considered the effect of diet on the individual microbial response. Necrotizing enterocolitis, a micro-organism-mediated disease, has a significantly higher incidence in very low birth weight infants fed formula rather than human breast milk [11–13]. One unaddressed question is whether this observation is based solely on variation in microbial populations selected for by the two diet classes, or whether diet can influence the virulence of the bacteria themselves. The latter mechanism may be possible via carbon catabolite repression, a process that links bacterial carbon metabolism to virulence gene expression. In carbon catabolite repression, bacteria presented with a preferred carbon source repress genes associated with the metabolism of secondary sources and thus conserve energy. In the absence of a preferred carbon source, bacteria must activate transcription of alternative metabolism pathways—a process that may result in upregulation of the expression of virulence factors to help digest non-preferred carbon sources [14–16]. Therefore, it is plausible that diet directly influences the pathogenicity of bacteria in the gut.

To investigate the poorly understood relations between diet and intestinal microbial phenotype, we examined the virulence response of *P. aeruginosa* to dietary monosaccharides. This organism is an opportunistic pathogen present in the gut of infants at risk for NEC [12,17] that alters its phenotype in response to local environmental cues (e.g., pH, metabolites, antibiotics, host factors) [18–24]. These characteristics, along with its extensively mapped and categorized genome, make *P. aeruginosa* an ideal organism for such an analysis. Monosaccharides were selected for our study on the basis of the work of Poroyko et al., which determined that infant formula can be distinguished easily from human milk based, in part, on its monosaccharide composition [25]. As a consequence, we hypothesized that *P. aeruginosa* would express virulence genes differently when exposed to monosaccharides present in formula versus those in human milk.

Materials and Methods

Overnight bacterial cultures

Overnight cultures of *P. aeruginosa* were prepared by plating a stock PAO1 strain on *Pseudomonas* Isolation Agar (PIA) (Sigma-Aldrich Corp., St. Louis, MO) and incubating at 37°C overnight. Isolating single colonies from PIA plates, inocula were added to 3 mL of tryptic soy broth (TSB) and grown overnight at 37°C with 180 rpm agitation.

Monosaccharide selection

The API 50 CH Research Strips from bioMérieux Clinical Diagnostics (Marcy l'Etoile, France) were used to identify carbohydrates metabolized by *P. aeruginosa*. Strip wells were inoculated as described in the kit instructions from a 3-mL overnight bacterial culture centrifuged at 3,000 rpm/23°C for 15 min and resuspended in 10 mL of CHB/E medium (Microbiologics, Inc., St. Cloud, MN). Inoculates were then incubated at 37°C and the results recorded at 24 and 48 h.

Growth curve and pyoverdine assay

Pyoverdine is a siderophore produced and secreted by *P. aeruginosa* that aids in the bacterium's acquisition of iron

from the host environment. Pyoverdine can be measured by fluorescence. Its production over time was monitored to identify the point at which different monosaccharide environments first influence the *P. aeruginosa* virulence response. Growth medium was prepared by supplementing TSB with selected monosaccharides at 0.5% and autoclaving for 15 min at 121°C. An overnight culture of *P. aeruginosa* was centrifuged at 5,000 rpm for 10 min, resuspended in 3 mL of fresh TSB, and added to the selected growth medium at a 1/100 dilution. Then 200-mL aliquots of inoculated medium were distributed to a black, 96-well clear-bottom assay plate from Corning Inc. (Corning, NY) and grown at 37°C. Cell density was determined as optical density (OD) at 600 nm using a 96-well Microplate Fluorimeter Plate Reader (Synergy HT, Biotek Corp., Broadview, IL). Pyoverdine production was determined by a fluorescence measurement at 400 ± 10/460 ± 40 nm excitation/emission using the same instrument. The relative fluorescence units (RFUs) were then normalized to the OD at 600 nm.

Biofilm assay

As with pyoverdine, biofilm formation was measured to identify the time at which the monosaccharide environment first influenced the *P. aeruginosa* virulence response. Cultures used for the growth curve and pyoverdine assay were aliquoted simultaneously and grown in clear 96-well plates. Biofilm formation was quantified using a protocol modified from O'Toole et al. [26]. After 6, 12, and 24 h of growth, a plate was emptied of its bacterial contents and washed three times with PBS. A 0.1% solution of crystal violet was added to each well and allowed to incubate for 15 min, after which the plates were submerged in deionized H₂O until excess crystal violet was removed. Once the wells were dry, biofilm-bound crystal violet was dissolved in 200 µL of 95% ethanol, of which 175 µL was transferred to a new 96-well plate and read at 590 nm using a 96-well Microplate Fluorimeter Plate Reader (Synergy HT, Biotek).

Quantitative reverse transcriptase-polymerase chain reaction primer array

A total of 59 *P. aeruginosa* virulence genes of interest were identified (Table 1). These genes, coding for key structural proteins, enzymes, or transcriptional regulators of virulence factor synthesis, were divided into 14 categories: Alginate, cyanide, flagella, proteases, pyochelin, pyocyanin, pyoverdine, quorum sensing, virulence regulation, rhamnolipids, toxins, type III secretion systems, type IV pili, and carbohydrate regulation. Alginate, a capsule polysaccharide, gives *P. aeruginosa* anti-phagocytotic and biofilm-forming properties [21,27], and cyanide inhibits mitochondrial enzymes involved in host cellular respiration [27]. Flagella, in addition to providing motility, contain proteins specific for adhesion to the mucin lining of the respiratory and gastrointestinal tracts [28–30]. Proteases, specifically the elastases, degrade mucin to provide substrates for bacterial metabolism [31]. Pyochelin and pyoverdine are siderophores produced and released by *P. aeruginosa* that chelate iron in the host environment and subsequently bind complex-specific receptors on the bacterium's outer membrane to allow iron uptake [32]. Pyocyanin, the pigment-producing virulence

TABLE 1. *PSEUDOMONAS AERUGINOSA* VIRULENCE GENES CODING FOR KEY STRUCTURAL PROTEINS, ENZYMES, OR TRANSCRIPTIONAL REGULATORS

	<i>Gene</i>	<i>Gene product</i>
Alginate	algU	Sigma factor (RNA polymerase subunit) AlgU
	mucB	Transcriptional regulator MucB
Cyanide	algD	GDP-mannose-6-dehydrogenase AlgD
	hcnB	Hydrogen cyanide synthase HcnB
Flagella	hcnC	Hydrogen cyanide synthase HcnC
	fliC	Flagellin type b
	fliD	Flagellar capping protein FliD
	fleQ	Transcriptional regulator FleQ
	fleR	Response regulator FleR
	fliA	Sigma factor (RNA polymerase subunit) FliA
	flgM	Anti-sigma 28 factor FlgM
Protease	aprA	Alkaline metalloproteinase precursor AprA
	lasA	Elastase LasA
	lasB	Elastase LasB
Pyochelin	fptA	Fe(III)-phochelin outermembrane receptor FptA
Pyocyanin	pchA	Isochorismate synthase PchA
	phzA2	Phenazine biosynthesis protein PhzA2
	phzM	Phenazine-specific methyltransferase PhzM
Pyoverdine	phzA1	Phenazine biosynthesis protein PhzA1
	pvdF	Pyoverdine synthetase F
	fpvA	Ferripyoverdine receptor
	pvdD	Pyoverdine synthetase D
Quorum sensing	pvdS	Sigma factor (RNA polymerase subunit) PvdS
	lasR	Transcriptional regulator LasR
	lasI	Autoinducer synthesis protein LasI
	rhII	Autoinducer synthesis protein RhII
Virulence regulation	rhIR	Transcriptional regulator RhIR
	Vfr	Transcriptional regulator Vfr
	gacA	Global activator & response regulator GacA
	rpoN	RNA polymerase sigma 54 factor
	cbrA	Two component sensor CbrAB
	cbrB	Two component sensor CbrAB
	rhIB	Rhamnosyltransferase chain B
Rhamnolipids	rhIA	Rhamnosyltransferase chain A
Toxins	plcB	Phospholipase C
	exoT	Exoenzyme T
	plcH	Hemolytic phospholipase C precursor PlcH
	toxA	Exotoxin A precursor
	exoY	Adenylate cyclase ExoY
	plcN	Nonhemolytic phospholipase C precursor plcN
	pldA	Phospholipase D
	exoS	Exoenzyme S
	rnr	Exoribonuclease RNase R
	pcrV	Type III secretion system protein PcrV
	popB	Translocator protein PopB
	popD	Translocator outer membrane protein PopD
Type III secretion system	exsA	Transcriptional regulator ExsA
	pscF	Type III export protein PscF
	pilG	Twitching motility protein PilG
	pill	Twitching motility protein Pill
	pilJ	Twitching motility protein PilJ
	pilK	Methyltransferase PilK
	chpA	Chemotactic signal transducer ChpA
Type IV pili	chpB	Chemotactic signal transducer ChpB
	pilA	Type IV fimbrial precursor PilA
	rhsR	Ribose operon repressor RhsR
	gntR	Transcriptional regulator GntR
	fruR	Fructose responsive transcription factor
Carbohydrate utilization	crc	Catabolite repression control protein

GDP = glutamyl depeptidase; RNA = ribonucleic acid.

factor of *P. aeruginosa*, is a reactive oxygen species (ROS)-generating factor that kills host cells and other bacteria and disrupts the proliferation and phagocytic functions of immune cells [33,34]. Quorum-sensing mechanisms allow the bacterium to regulate its activity according to its environment [35], and the global virulence regulators influence the expression of multiple virulence factors across categories [36]. Rhamnolipids are biosurfactants that take part in the development of biofilms and in swarming motility [33]. Toxins secreted into the extracellular space by *P. aeruginosa* act via different mechanisms to achieve end effects such as DNA synthesis inhibition, cytoskeleton disruption, and phagocytosis evasion [32]. Three toxins—ExoS, ExoT, and ExoY—are introduced directly into the host cell via a translocation pore formed by the cellular machinery of type III secretion systems [37]. Finally, type IV pili are central to chemotaxis and adhesion [38,39], and carbohydrate regulation proteins play a role in carbon catabolite repression [32]. Specific primers for these 59 genes were designed using a BLAST search of the *Pseudomonas* Genome Database [32,40]. The specificity of all primer pairs was verified with a single melting curve.

Quantitative reverse transcriptase-polymerase chain reaction

To determine the differential expression of virulence genes in *P. aeruginosa* grown with select monosaccharides, 10 mL of medium was inoculated as described for the growth curve and pyoverdine assay. Cultures were incubated aerobically for 6 or 24 h at 37°C with 180 rpm agitation. The cultures were then stabilized with RNAprotect

Bacteria Reagent (Qiagen, Hilden, Germany) and the organisms pelleted by centrifugation at 5,000×g. Total bacterial RNA was extracted and treated with DNAase using the RiboPure Bacteria Kit (Ambion, a division of Life Technologies, Inc., Carlsbad, CA). The RNA quality and concentration was estimated by Nanodrop (NanoDrop Technologies, Inc., Wilmington, DE). Then 2 mcg of total RNA was mixed with random primer and Superscript III (Invitrogen, a division of Life Technologies) for a 2-h reverse transcription reaction. The cDNA product was diluted 1:20 in nuclease-free water for use in quantitative polymerase chain reaction (qPCR). Each 10-mL qPCR reaction mixture contained 0.4 mM of each primer, 2×SYBR Green Master Mix (Applied Biosystems, a division of Life Technologies), and 0.4 mL of the diluted cDNA template. The qPCR was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems).

Data analysis

The extent of virulence gene expression was calculated from differences in Ct values, an output of the 7900HT Fast Real-Time PCR System (Applied Biosystems). We found that expression of pchF (PA4225) was not influenced by the monosaccharide environment; thus, this gene was used to normalize expression of all genes. The normalized value for the control—*P. aeruginosa* grown aerobically in TSB alone—was subtracted from each experimental value and the result inserted as $\Delta\Delta C_t$ into the equation $n = 2^{-(\Delta\Delta C_t)}$. This calculation provided the fold difference in gene expression of our experimental set in relation to the control.

FIG. 1. View of API 50 CH Research Strips showing *Pseudomonas aeruginosa* sugar metabolism after incubation at 37°C. Color change indicates metabolism. (A) Glycerol (GLY), arabinose (ARA), ribose (RIB), xylose (XLY), galactose (GAL), glucose (GLU), fructose (FRU), mannose (MNE), and mannitol (MAN) all show evidence of metabolism after 24 h. (B) After 48 h, only ARA, RIB, XYL, GAL, and MNE showed signs of continued metabolism, and of these, the four marked * distinguish infant formula from breast milk diets.

Results

P. aeruginosa PAO1 selectively metabolizes dietary monosaccharides

As indicated by a red to yellow color change in the API 50 Research Strips (bioMérieux Clinical Diagnostics) *P. aeruginosa* metabolizes a variety of substrates after 24 h of aerobic growth at 37°C (Fig. 1A). However, because the yellow color is produced by the interaction of substrate metabolites with components of the kit medium, it does not indicate whether the substrates are being metabolized or have already been consumed at the 24-h time point. Interestingly, several substrates metabolized within 24 h continued to produce the yellow color at 48 h (Fig. 1B). These substrates presumably are still being metabolized at that time.

Of the substrates demonstrating active metabolism at 24 h, four are monosaccharides specific to either milk-fed or formula-fed infants, as determined by Poroyko et al. [25]. Specifically, arabinose is a metabolic marker of infant formula, xylose a marker of human breast milk, galactose a marker of both formula and the feces from milk-fed infants, and mannose a marker of feces from milk-fed infants. Accordingly, these four monosaccharides were selected for use in subsequent studies of virulence gene expression.

Monosaccharides influence pyoverdine production and biofilm formation in a 24-h *P. aeruginosa* culture

Monosaccharides had no effect on pyoverdine production or biofilm formation during the early stages of culture growth (Fig. 2B, C) when corrected for the slight differences in growth rate (Fig. 2A). Given adequate time to deplete the preferred nutrients in TSB and ultimately to metabolize the added sugars, *P. aeruginosa* demonstrated a unique response to its monosaccharide environment. At 24 h, the bacteria grown in the presence of mannose consistently produced less pyoverdine and biofilm components than those grown in the presence of other monosaccharides or in TSB alone (Fig. 2B, C).

Virulence Gene Expression of *P. aeruginosa* Grown in Select Monosaccharides

Figure 3 displays the log₁₀ of the fold difference in virulence gene expression for *P. aeruginosa* grown in monosaccharide-supplemented TSB in comparison with bacteria grown in TSB alone. Positive values indicate gene up-regulation and negative cell values down-regulation. Furthermore, the cells were formatted conditionally so that the extent of gene up-regulation and down-regulation corresponds to the intensity of the red and green color, respectively. Supporting the preliminary observations of pyoverdine and biofilm production, *P. aeruginosa* did not respond differentially to monosaccharides after 6 h of growth. At this time, gene expression in the experimental groups was similar to that of the TSB control. After 24 h, however, monosaccharides demonstrated an effect on *P. aeruginosa* virulence gene expression. Arabinose and galactose, markers of infant formula and both infant formula and the feces of breast milk-fed infants, respectively, caused extensive up-regulation across all categories of virulence genes. Xylose, a marker of human breast milk, caused a slight attenuation of virulence gene expression compared with the previous two sugars. Mannose, a marker in the feces of breast milk-fed infants, led to extensive attenuation of the virulence gene response by

FIG. 2. Aerobic response of PAO1 to arabinose (◆), galactose (▲), mannose (X), xylose (●), and tryptic soy broth control (■) as measured by (A) growth rate, (B) pyoverdine production (shown in relative fluorescence units normalized to optical density), and (C) biofilm formation.

P. aeruginosa, with expression of several categories of virulence genes falling to less than that of the control organisms.

Discussion

P. aeruginosa displayed a time-dependent response to monosaccharides when grown under aerobic conditions. Whereas 6 h of growth failed to elucidate any influences of sugar on *P. aeruginosa* virulence, 24 h of growth was associated with marked up-regulation of virulence by two of the four monosaccharides studied. That is, arabinose and galactose increased expression in all 14 virulence gene categories measured. Xylose also increased virulence gene expression in all 14 categories but to a lesser extent than either arabinose or galactose. Mannose increased gene expression extensively in only four categories—alginate, flagella, quorum sensing, and type IV pili—while attenuating the virulence response in the remaining 10. With arabinose being a distinguishing component of formula, xylose a distinguishing component of milk, mannose a distinguishing component of feces from milk-fed infants, and galactose a distinguishing component of formula

FIG. 3. Quantitative reverse transcriptase polymerase chain reaction data for *Pseudomonas aeruginosa* PAO1 grown with select monosaccharides for 6 and 24 h. Data have been formatted to represent the log of the fold difference in gene expression between the experimental and control (*P. aeruginosa* grown in tryptic soy broth alone) groups. Intensity on right correlates with magnitude of gene up-regulation, and intensity on left correlates with magnitude of gene down-regulation.

and feces from milk-fed infants, it appears that not all components of milk contribute to its reported protective effects, as two milk-associated monosaccharides encouraged *P. aeruginosa* to express a more virulent phenotype.

Although data from the present study were not able to confirm a discriminative value of dietary monosaccharides on microbial virulence per se, significant insight into the process was generated. Similar to the way staphylococci and other gram-positive microorganisms regulate virulence factor synthesis according to their metabolic state [16], it appears that *P. aeruginosa* controls its virulence by responding to its carbon microenvironment. The organism's preferred carbon source is not sugar; rather, it favors amino and organic acids such as succinate, pyruvate, and acetate [41,42]. Therefore, after 6 h of culture, when the bacterial density is low and the preferred casein and soy meal peptones of TSB are plentiful, *P. aeruginosa* turns off the metabolic pathways used in the digestion of sugars and concentrates its resources on the preferred source. However, at 24 h, when the bacterial

density is high and the preferred nutrients in TSB have been depleted, *P. aeruginosa* shifts to a secondary carbon source consisting of monosaccharides. At this time, *P. aeruginosa* up-regulates its virulence gene complement, potentially using these virulence factors to scavenge for a new carbon source [43].

This relation between nutrient availability and virulence may explain the higher incidence of inflammatory diseases of the gut associated with certain diets. With differences in the relative abundance of metabolite classes and in the individual metabolite composition within each class, each diet provides a unique balance of preferred and non-preferred carbon sources. In addition to selecting for the growth of a bacterial population, this carbon balance has the potential to influence the phenotype of the residing bacteria. Supplied with the right diet, bacteria may be provided with a sufficient amount of preferred carbon sources, leading to the expression of a more benign phenotype. Conversely, alternative diets may shift the balance in favor of non-preferred

carbon sources, thus allowing bacteria with a more virulent phenotype to emerge.

Although the results of the present study suggest that *P. aeruginosa* responds independently to its monosaccharide microenvironment, they do not account for several other factors that likely contribute to the virulence response. First, *P. aeruginosa* is a strictly aerobic microorganism, usually contributing to intestinal disease following an opportunistic infection. For monosaccharides to have an effect during such infections, *P. aeruginosa* would have to reside in the intestine for an extended period of time, both resisting expulsion and competing successfully with native flora [44]. Such extended survival could be accomplished if *P. aeruginosa* used nitrate as a final electron acceptor to thrive anaerobically. However, this hypothesis raises the question of whether nitrate modifies the overall metabolism of *P. aeruginosa* independent of the carbon microenvironment. Second, the present study subjected *P. aeruginosa* to one monosaccharide at a time. Accounting for relative metabolite abundance in vivo, it is possible that mannose predominates in the intestine of milk-fed infants, resulting in an overall reduction in *P. aeruginosa* virulence despite the opposing influence of galactose or xylose. Finally, in addition to monosaccharides, the infant microbiome receives carbon in the form of organic and amino acids, sugar alcohols, and fatty acids. Although these alternative carbon sources are more homogenous than sugars in composition in breast milk- and formula-fed infants [25], they could still play a central role in the virulence response, given *P. aeruginosa*'s demonstrated preference for amino and organic acids [41,42]. Furthermore, attenuation of the virulence response may be seen in milk-fed infants because of the presence of galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS). Along with promoting bifidogenicity and its associated protective qualities, GOS and FOS may act directly on pathogenic bacteria by mimicking eukaryotic cell-surface receptors and preventing adherence of virulent bacteria [5–7]. Additionally, these oligosaccharides undergo fermentation in the gut and produce both short-chain fatty acids (SCFA) and hydrogen ions [5,7]. Such products are likely to attenuate the virulence response further by altering the nutrient content and pH of the surrounding milieu [7], and, in the case of SCFA, by decreasing colonic permeability and acting as an anti-inflammatory mediator [5,45].

Even if the monosaccharide composition of infant diets does play a substantial role in the virulence of *P. aeruginosa*, it remains unclear if diet has this same influence on other gut bacteria. As oxygen is consumed by aerobic microbes in the early, rapidly evolving infant gut, anaerobes gain a competitive advantage and construct a flora with greater stability [4,46]. Although reports have linked both aerobes and anaerobes to intestinal inflammatory disease such as NEC [12], such native anaerobes may display a different set of responses than the ones observed with our model microorganism. Nonetheless, it is likely that these native anaerobes also demonstrate virulence expression that is responsive to diet. Numerous observational studies have implicated both the microbiota and diet as mediators of inflammatory bowel disease [45,47], a disease that characteristically presents in early adulthood when one's intestinal flora has been established with commensal anaerobes. Investigators also have isolated *Escherichia coli* from patients with recurrent Crohn disease and demonstrated up-regulation of certain genes central to virulence and survival

[48]. Whether it is the components of the diet that lead to these changes remains unclear.

In conclusion, these preliminary studies demonstrate the importance of nutrient content on the relative expression of virulence genes in pathogens that colonize the gut of infants commonly. Using genomic and deep proteomic/metabolomic analysis to understand the effect of current dietary formulas on virulence gene expression across such gut-colonizing pathogens could advance our understanding of the differences between human milk and formula in the development of NEC and other mucosal inflammatory diseases.

Acknowledgments

This work was supported by National Institutes of Health grant 5 R01 GM62344-11 (to JCA) and the University of Chicago Pritzker School of Medicine Summer Research Program (NIH).

Author Disclosure Statement

No competing financial interests exist.

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