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Human flora-associated rodents – does the data support the assumptions?

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Summary

There is little direct literature detailing exhaustive bacteriological studies comparing human donor faecal flora, human flora-associated (HFA) mouse models and conventional rodent faecal flora. While there is a premise that the implanted donor faecal flora from humans is established in the rodent model the evidence is incomplete and indeed for groups such as *Bifidobacterium* spp. it is lacking. The reviewed bacteriology studies are generally lacking in detail with the exception of one study from which the data have mostly been overlooked when cited by other workers. While there are studies that suggest that the HFA rodent model is more relevant to man than studies with conventional rodents, the hypothesis remains to be proven. This review concludes that the established microbial flora in the HFA rodent model is different to that of donor human faecal flora, and this clearly raises the question as to whether this matters, after all a model is a model and as such models can be useful even should they fail to be a true representation of, in this case, the gastrointestinal tract. What matters is that there is a proper understanding of the limitations of the model as we attempt to unravel the significance of the components of the gastrointestinal flora in health and disease; examples of why such an analysis is important are provided with regard to obesity and nutritional studies. The data do unsurprisingly suggest that diet is an extremely influential variable when interpreting HFA and conventional rodent data. The microbiology data from direct bacteriology and indirect enzyme studies show that the established microbial flora in the HFA rodent model is different to that of donor human faecal flora. The significance of this conclusion remains to be established.

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Introduction

It is accepted that the adult human being consists of approximately ten times as many microbial as mammalian cells and that about 1.25 kg of microbes is carried by the average adult. The complex indigenous microbial flora plays a role in protecting man against pathogenic organisms, contributes to our energy and vitamin supply and is pivotal in the development of our immune system. It is because of this relationship that microbiologists have for many years endeavoured to establish appropriate methods of study to elucidate this symbiosis. Rumney and Rowland (1992) reviewed the then available models and listed the numerous methodological challenges to studying the ecology and metabolism of the colonic flora of man and concluded that there appears to be no single ideal approach. They identified problems that included the difficulty of working with a strictly anaerobic population; problems in sampling colon contents; the fact that often used faecal flora may not be representative of colonic flora, variable effects of diet within human subjects, the inability to carry out toxicology studies for ethical reasons and the fact that the flora becomes disturbed when removed from the biotic and abiotic constraints of the human gut. The use of a human flora-associated (HFA) rodent model, whereby human flora is implanted into gnotobiotic rodents, which are subsequently maintained in a facility that protects them from being exposed to exogenous flora, was an attempt to circumvent some of these challenges. This approach allows for a level of dietary, environmental and genetic control by maintaining the microbial flora in an *in vivo* environment similar to that of the human gastrointestinal tract. The HFA rodent model has and continues to be used to study the relationship between the human gut flora and health and diseases such as stomach and lower bowel cancer and inflammatory bowel disease, as well as in a whole range of toxicology studies.

There appear, however, to have been few studies considering the relevance of *in vivo* HFA rodent models that have considered the assumptions that lie behind their use. It is tacitly assumed that the HFA rodent model does indeed mimic the flora of the human gastrointestinal tract and that the animal model will therefore be predictive of the situation in man. This paper reviews the literature with regard to examining direct and indirectly measured

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differences between the bacterial flora of HFA rodents, conventional rodents and man to establish the microbiological basis of the use of HFA rodents as a surrogate for studying the effects on the human gastrointestinal flora. It is pertinent to make clear at the outset that there are fundamental differences between the gastrointestinal tract of rodents and man apart from the questions of bacterial flora, such differences are not addressed in this paper. This review concludes that the established microbial flora in the HFA rodent model is different to that of donor human faecal flora, and this clearly raises the question as to whether this matters, after all a model is a model and as such models can be useful even should they fail to be a true representation of, in this case, the gastrointestinal tract. What matters is that there is a proper understanding of the limitations of the model as we attempt to unravel the significance of the components of the gastrointestinal flora in health and disease. The role of the indigenous microbiota has been eloquently reviewed by Tannock (2008), and two examples will be described that highlight why it is important to understand the limitations of the HFA rodent model.

The first is taken directly from Tannock (2008) who made the point that molecular studies of the relationship between bowel bacteria and the mouse suggest that microbes impact importantly on murine physiology. *Bacteroides thetaiotaomicron* in the bowel, in relatively short-term gnotobiotic mouse experiments, influenced fucosylation of the enterocyte extracellular matrix, and upregulated or downregulated murine genes whose products are associated with nutrition, epithelial integrity and angiogenesis (Hooper *et al*., 1999; 2001; Stappenbeck *et al*., 2002). While this impacts on bowel mucosa, Tannock (2008) makes the point that there is also a systemic effect on the deposition of fat in relation to the bowel microbiota of mice. The latter work revealed that murine physiology is actually attuned to the downregulation of fat storage after weaning, but that the bowel microbiota negates the mouse regulatory mechanism by reducing the production of fasting-induced adipose factor (FIAF) in the intestinal mucosa that leads to a corresponding increase in the systemic activity of lipoprotein lipase (LPL). FIAF is an inhibitor of LPL, the latter influencing the uptake of triglycerides by adipocytes (Backhed *et al*., 2004). Tannock (2008) makes the point that it is unknown whether this phenomenon is applicable to humans. The issue here is that we do not yet understand which are the critical components of the gastrointestinal flora that are implicated in such interactions, and as such it is important that we are aware of the limitations of the models used.

The second example relates to the role of bifidobacteria; they are considered to be a significant bacterial population within the human gastrointestinal tract and play a pivotal role in health by maintaining a well-balanced intestinal microbiota (Reuter, 2001; Vaughan *et al*., 2005). Delgado *et al*. (2008) described the beneficial effects attributed to bifidobacteria to include the establishment of a healthy microbiota in infants, the prevention and treatment of diarrhoea, the alleviation of constipation and the symptoms of lactose intolerance, the enhancement of immune functions, cholesterol reduction and the suppression of tumorigenesis, among others (Ouwehand *et al*., 2002; Leahy *et al*., 2005). This genus is certainly of commercial relevance as most of the microbial components of commercial probiotics belong to the bifidobacterial group (Yeung *et al*., 2002; Fasoli *et al*., 2003). The reviewed data suggests that this important group of organisms is not always implanted in the HFA rodent model.

Basis for the HFA model

Silvi and colleagues (1999) in a study investigating the modification of gut flora by resistant starch stated that,

> The study was performed in human flora-associated rats, which provide greater relevance to man than using conventional flora rats. Human flora-associated rats, obtained by colonizing germ-free rats with human faecal bacteria, develop and maintain a flora with bacteriological and metabolic characteristics similar to that of the mature human colonic microflora.

The authors cited the work of Mallett and colleagues (1987), Hirayama and colleagues (1995) and Rumney and Rowland (1992) as support for this far-reaching statement and one that has been used many times as justification for the HFA model. It is necessary to closely examine these source references in some detail.

Mallett and colleagues (1987) compared gastrointestinal enzyme activities (β-glucosidase, β-glucuronidase, nitrate reductase and nitroreductase) in conventional and HFA rats and human faeces. They concluded that conventional and HFA rats exhibited comparable enzyme activities that were similar to those found in human faeces with the exception of nitrate reductase, which exhibited negligible activity in conventional rats. The conventional rat flora did, however, respond differently to HFA rats and to human faeces when diet was supplemented with pectin in that nitrate reductase activity was stimulated. Evidence for the similarity of flora between the HFA rodent model and man was thus based not on direct bacteriological data but on similarity in enzyme activities of three of the four tested enzymes.

As a prelude to the cited study of Hirayama and colleagues (1995), it is important to acknowledge that Hirayama and colleagues (1991) demonstrated that most of the major components of human faecal bacteria could be transferred into HFA mice, although this was not true

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for the important *Bifidobacterium* group, which were not established in the model. Indeed, the authors suggested that this could be a model to study the implications on human health of this important group of bacteria. This initial study was followed by a comprehensive study comparing the composition and metabolism of faecal microbiota among 'human flora-associated' mice inoculated with faeces from six different human donors (Hirayama *et al*., 1995). It was this latter study that was cited by Silvi and colleagues (1999). It is probably the most comprehensive of all studies with regard to the detailed bacteriology that was carried out and is worth considering in some detail. The bacterial composition of the microbiota, bacterial reductive and hydrolytic enzyme activities, concentrations of short chain fatty acids (SCFAs) and putrefactive metabolites in faeces of germ-free mice inoculated with faecal suspensions of six different volunteers were examined. The study confirmed that human faecal bacteria could be transferred into the intestine of HFA mice, although there were differences between the human donor inoculum and the established flora. In HFA mice, the numbers of *Eubacterium* spp., anaerobic cocci and *Enterobacteriaceae* were significantly higher than those in conventional mice but similar to those in man. The numbers of total bacteria, *Bacteroides* spp., *Clostridium* spp. and *Streptococcus* spp. were significantly higher than those in humans and conventional mice. Perhaps of greatest significance is that bifidobacteria were eliminated from three out of the six HFA mouse groups. Despite these differences, which are clearly detailed in the *results* section of the paper, the discussion merely states that, 'faecal bacteria could be transferred into the intestine of HFA mice with minor modification, except for a decrease or elimination of bifidobacteria in one half of the HFA mouse groups'. The abstract to the paper simply says, 'The composition of major faecal bacteria of HFA mice was similar to that of inoculated human faeces, although bifidobacteria were eliminated from some HFA mouse groups'. It is clear that unless the paper is read in some detail, the reader could easily draw the wrong conclusions.

The authors make the point that the reasons for the differences between donor flora and established flora are not clear. Regardless of inoculated human faeces, the numbers of *Bacteroides* spp., *Clostridium* spp., anaerobic cocci and *Streptococcus* spp. in all HFA mice were higher than those in the inocula. Hirayama and colleagues (1995) suggest that the bacterial balance in the intestine of the HFA mice might be controlled by physiological conditions of the mouse intestine, rather than by the balance of the microbes in the human faeces. This will, as they point out, reduce the variability in bacterial composition among HFA mouse groups when compared with what happens in human faecal samples.

The activities of β-glucosidase and β-glucuronidase of HFA mice were similar to those in man but the activities of nitrate reductase and nitroreductase in HFA mice were different from those in both man and conventional mice. Additionally, the concentrations of putrefactive products in the faeces of HFA mice were largely different from those in human faeces but similar to those found in conventional mice. The composition of faecal SCFAs in HFA mice was more similar to that in humans than that in conventional mice, but the acid concentrations were significantly lower than those found in man. Bacterial metabolism in the intestine of HFA mice thus reflected that of human faeces with respect to some metabolic activities but not others. The authors suggest two hypotheses by which to explain the results:

(i) dominant bacterial species of each bacterial group in HFA mice might change from those present in inoculated human faeces; and
(ii) bacterial metabolism might change because of an altered intestinal environment.

Further comment was made that the variations of composition and metabolic activities of the faecal microbiota among HFA mice groups were not particularly obvious, and perhaps more importantly the individual variations among inoculated human faeces were not reflected in HFA mouse groups and that some of the characteristics of each human donor faeces were hardly reflected in the HFA mice.

While this paper is widely cited as justification for the use of HFA rodents as models for study of the gastrointestinal tract flora in man, there appears to have been no close scrutiny of the data nor even the cautionary final sentence in the abstract:

> These findings indicate that HFA mice provide a stable and valuable tool for studying the ecosystem and metabolism of the human faecal microbiota, but they have some limitations as a model.

The final supportive reference mentioned above was from a review carried out by Rumney and Rowland (1992). Within the review they cited the work of Hazenberg and colleagues (1981), who through a detailed bacteriology study showed that the gross bacterial composition of the HFA mouse was similar to that from man and distinct from that of a conventional mouse. These workers further demonstrated that the caecum of the HFA mouse was much reduced in size to a level that was equivalent on a body weight basis with that of man. The differences between the HFA mouse flora and that of the conventional mouse were easily demonstrated in that they reported a replacement of the human flora by murine flora within 2 weeks if the mice were not retained in their respective isolators. This is a rather important point in that it suggests that the human flora is not really stabilized in

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the mice, and that it could be more readily perturbed by antibiotic exposure than would be the case in the human gastrointestinal tract.

Ducluzeau and colleagues (1984) confirmed the work of Hazenberg and colleagues (1981), and according to Rumney and Rowland (1992) this was also confirmed by Pecquet and colleagues (1986). Close reading of the Pecquet and colleagues (1986) study, however, reveals that while they showed similarity in flora between HFA mice and human volunteers this was only with respect to *Enterobacteriaceae* and total anaerobes, no other groups were tested nor was there any comparison with the flora from conventional animals. Another error in the Rumney and Rowland (1992) review occurs in their citation of the previously discussed paper from Mallett and colleagues (1987). The review concludes from the Mallett and colleagues (1987) paper:

> When the HFA animals were compared with their conventional flora counterparts all four microbial enzymes studied showed significant differences in activity.

Examination of the Mallett and colleagues (1987) paper as described previously shows this not to be the case. The Rumney and Rowland (1992) review also presented some previously unpublished data showing that the metabolism of 2-amino-3-methyl-3H-imidazo[4,5-f] quinoline occurred faster in bacterial suspensions isolated from rat and mouse faeces than from human faeces yet the rate of reaction from HFA rat faeces was similar to that from human faeces. Ward and colleagues (1990) in a similar study investigated the effect of different types of dietary fat on the formation of *N*-nitro-soproline in germ-free, conventional and HFA rats. The results in HFA rats and humans were similar and contrasted with those in conventional rats.

What is clear is that the evidence supporting the view that HFA rat studies are more relevant to the situation in man than studies with conventional rats (Silvi *et al*., 1999) is not as substantial as might otherwise be thought. The studies of Hirayama and colleagues (1991; 1995) are the only exhaustive supporting studies, which have carried out a thorough bacteriological analysis. There are a number of studies that provide indirect support for HFA rodent models being predictive of the situation in man with regard to GI tract metabolism. Rowland and Tanaka (1993) described the feeding of transgalactosylated oligosaccharides (TOS) to HFA rats resulting in an increase in total anaerobe, *Bifidobacterium* and *Lactobacillus* spp. counts but not for *Bacteroides* spp. These results were consistent with those published by Ito and colleagues (1990), who fed TOS to human volunteers and found significant increases in *Bifidobacterium* spp. and *Lactobacillus* spp. in the faeces, although there were no changes in numbers of total bacteria.

Mallett and colleagues (1987) examined the influence of incubation pH on the activity of rat and human gut flora enzymes (β-glucosidase, β-glucuronidase and nitrate reductase). All three enzymes were influenced by pH, as exemplified by β-glucosidase activity that diminished as pH increased. In other instances the rat and human flora showed distinct profiles with nitrate reductase activity undetectable in human faeces below pH 6.6, whereas the rat caecal flora displayed optimal reduction of nitrate around neutrality. The most pronounced host-species difference was found with β-glucuronidase. The authors considered that the diverging profile for the two sources of intestinal material may reflect differences in the occurrence of β-glucuronidase-positive organisms present in the rat or human gut, as different bacterial species possess widely varying levels of this enzyme (Cole *et al*., 1985).

Apart from the study of Hirayama and colleagues (1991; 1995) there appear to be few such comprehensive studies considering comparative bacteriological analysis of the faecal microbiota of conventional, HFA rodents and donor human faeces. There are, however, other studies in the literature that provide pointers as to differences between the faecal microbiota of conventional rodents, HFA rodents and man.

It has become possible to analyse the complex concentrated anaerobic bacterial fraction of the gut microbial flora by a technique known as micromorphometry. This technique utilizes sophisticated digital image processing that enables detection and quantitative as well as qualitative analysis of bacterial objects in faeces, based on their morphological appearances. Analysis is determined by the distribution of bacterial species present in faeces without dilution of the sample. Using this technique, Veenendaal and colleagues (1996) showed that the micromorphological patterns of faecal anaerobes from HFA mice were different to those from ex-germ-free mice associated with mouse-specific pathogen-free flora. In particular, the authors studied germ-free mice associated with either related rodent SPF microflora (SPF-MF) or unrelated human microflora (HUM-MF). The micromorphological pattern of the bacteria from faeces of HUM-MF-associated mice was significantly different from SPF-MF-associated mice. There was interestingly gross morphological variability between individual HUM-MF mice but not between the individual SPF-MF mice. No differences were found between the donor human flora and the donor flora from the SPF mice. There was a high degree of variability in the conventional mouse flora. While not citing any supporting data the authors, in the introduction, made the statement that, 'The highly concentrated anaerobic fraction in rodents is known to be micromorphologically different compared with those in humans, e.g. stool specimens of mice contain large

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numbers of fusiform shaped bacteria whereas small rod shaped and coccoid bacteria are predominant in human faeces'. The findings from their reported study appear to conflict somewhat with this statement. Koopman and colleagues (1989) and Veenendaal and colleagues (1988) have similarly described differences in faecal flora between different rodent species.

As far back as 1980 Raibaud and colleagues (1980) carried out a series of fascinating experiments in which bacteria from the digestive tract of man and various animals were implanted into gnotobiotic mice. In the first experiment fourteen microbial strains isolated from conventional rats were inoculated into axenic rats and mice receiving identical diets. The populations of these organisms, which became established in the faeces of the gnotobiotic adult recipient rats and mice, were quite similar apart from one strain of *Clostridium* sp., which established in rats but not the mice. The complex flora from a conventional chicken was then successfully implanted in adult gnotobiotic mice such that it exhibited an intact barrier effect against *Salmonella typhimurium*. Finally Raibaud and colleagues (1980) inoculated faeces from a human donor into adult gnotobiotic recipient mice. Colonization was achieved although members of the genera *Bifidobacterium, Lactobacillus* and *Clostridium* were not able to establish despite being present in the donor inoculum. Nevertheless the colonizing flora did still exert an effective barrier against a toxigenic strain of *Clostridium difficile*, although this barrier effect spontaneously disappeared several weeks later.

García-Lafuente and colleagues (2001) working with pathogen-free Sprague-Dawley rats have clearly shown that the colonic barrier function is modulated by the composition of the commensal GI tract flora. Colonization of the colon by *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus viridans* was seen to compromise colonic barrier function whereas colonization with *Lactobacillus brevis* reduced colonic permeability and thus improved barrier function.

Other experimental studies have shown that certain strains of lactobacillus may prevent or reverse intestinal permeability disorders. Prolonged cow's milk challenge in suckling rats increases gut permeability to intact protein and oral bacteriotherapy with *Lactobacillus casei* strain GG counteracts the increase in intestinal permeability (Isolauri *et al*., 1993). What is clear from these studies is that the commensal bacteria can influence the colonic barrier function in normal non-pathological conditions and that the interaction between the colonic flora and the gut wall can also be important. This last mentioned point is particularly important with regard to species-specific attachment mechanisms whereby many *Lactobacillus* spp. will exhibit host-specific attachment. The reason for highlighting these findings is that failure of one bacterial group to colonize a model may prejudice the results. Our current state of knowledge does not allow us to be sure of the importance of the respective bacterial groupings. There is general acceptance, however, that *Bifidobacterium* spp. are an important group within the gastrointestinal tract ecosystem yet they are a group that consistently fail to become established in the HFA model.

Differences between germ-free and conventional rodents

The epithelial structure of mammalian intestinal mucosa reflects the absorptive and secretory activity of its various segments. The apical surface membrane of intestinal epithelial cells has a well-defined layer of glycoproteins and glycolipids, the glycocalyx, which is involved in key cellular processes, such as adhesion, antigenic recognition, differentiation and transport. In an elegant study investigating the influence of diet and gut microflora on lectin binding patterns of intestinal mucins in rats Sharma and Schumacher (1995) described an overall reduction in sialic acid-linked D-galactose residues in conventional compared with germ-free rats and a loss of crypt-to-surface gradient of fucosyl expression in the large intestine of HFA rats. These differences were considered to be due to differing strains of glycosidases in the two different floras.

In a further study, Sharma and colleagues (1995) reported that while the effects of the human flora on the gut structure and mucus composition of HFA rats were in many ways similar to conventional rats, there were differences notably in the composition of the surface and goblet mucus and the length of the large intestinal crypt cells. While the authors acknowledge that these results may reflect real differences in response to the two types of flora they also consider it possible that when a human flora is inoculated in the germ-free animal, its establishment and subsequent interaction with the intestinal tract may induce changes not seen with an indigenous flora where colonization is not subject to experimental manipulation.

Taché and colleagues (2000) investigated the effect of carrageenans as potential tumour-inducing agents in conventional and HFA rats. At high dose levels in conventional rats aberrant crypt foci were detected whereas in contrast no effects were observed in HFA rats. These findings supported the hypothesis that it is the gut microflora of the host animal that is involved in the toxic effects of carrageenans in the rat colon.

Studies comparing metabolism in HFA rodents and man

Andrieux and colleagues (1993) investigated inulin fermentation in germ-free rats associated with a human intestinal flora from methane and non-methane producers. The donor human flora in both cases retained their fermentative properties when inoculated into germ-free

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rats fed control diet. Bacterial floras from human methane producers only gave rise to methane production, whereas hydrogen production was similar in both methane and non-methane-producing rats. Caecal SCFA concentrations were lower in methane-producing rats than in non-methane producers as was found in human faeces, but the lactic acid concentration was higher as compared with that in non-methane-producing rats.

Hirayama (1999) concluded that ex-germ-free animals harbouring intestinal microbiota originating in other animal species provide a stable and valuable tool for studying the ecosystem and metabolism of human faecal microbiota, but they have some limitations as a model. Hirayama believes that further studies concerning the composition of intestinal bacteria of HFA mice at the species level (NB *Bifidobacterium* spp.) and differences in intestinal physiological conditions among human, domestic animals and mice are needed. He further highlighted the findings of Rumney and colleagues (1993), who reported that the metabolic activities of intestinal bacteria of HFA rats appeared to be dependent on diet and that the results obtained with HFA rats were most relevant to humans when the animals were fed a human diet. Hirayama (1999) clearly stated that the development of a special diet for HFA mice is required to establish a better model to study the metabolism of human intestinal microbiota. Hentges and colleagues (1995) has similarly shown that diet is important when interpreting results in that gnotobiotic mice associated with human infant intestinal microorganisms were more resistant to colonization with *S. typhimurium* when consuming human milk than when consuming bovine milk, cows' milk-based formula or formula modifications.

No questions?

In the absence of hard microbiological data one wonders why so many workers simply cite the work of the pioneers of these models claiming the utility without describing the limitations; is this merely because they simply read abstracts and not the details of published papers or more likely because they have access to a model yet themselves have made no attempts to validate the test system? Perrin-Guyomard and colleagues (2001) used the HFA model to evaluate residual and therapeutic doses of tetracycline and apply this to impacts upon the human gastrointestinal flora. They based this on the fact that the model, 'may have high relevance in determining the effects of low levels of antibiotics on human microflora' and in this context cited studies of Corpet (1980; 1987), yet they did state that the model needed validation but did not appear to make any attempts to validate nor critically evaluate the model but rather reported data that they claimed related to human safety of antimicrobial residues. Indeed, they did not report how the bacterial counts related the donor counts but rather presented data showing the stability of the bacterial populations from 4 days post implantation. Where they did present comparative data for enzyme activities and volatile fatty acid production there were clear differences between the data from human and HFA samples, yet they concluded that the model was acceptable for studying dose-related effects of tetracycline on human intestinal flora. The same group Perrin-Guyomard and colleagues (2001) again used the model to address residue and therapeutic doses of ciprofloxacin on human gastrointestinal flora and, despite showing significant differences between bacteriological counts of the implanted and human flora, suggested that the model was appropriate for regulatory decision making.

This paper is not the first to question the validity of the HFA model. Wong and colleagues (1996) stated, 'The adequacy of a human faecal microbiota associated mouse as a model for studying the activities of human intestinal microorganisms was examined. During a 6 month period, several predominant aerobic and anaerobic components of the human faecal bacteria persisted at stable numbers in the intestinal tracts of mice. However, *Bacillus* species and both aerobic and anaerobic *Lactobacillus* species disappeared within 7 days after association. An inverse relationship existed between the presence of short-chain fatty acids and non-fatty organic acids in the caeca of the associated mice. The relative concentrations of the two acid groups changed over a 21 days period, suggesting an alteration in the pattern of metabolism by the bacteria during the course of study. The total amount of organic acid produced by the microorganisms in the caeca of the associated mice was approximately 25% of the published value for humans, suggesting that the human microbiota retained only a portion of its metabolic activities in the mouse host. When challenged orogastrically with *S. typhimurium*, associated mice were as resistant to colonization as conventional mice, but germ-free mice were very susceptible. Furthermore, Kibe and colleagues (2005) while using HFA pointed out the limitations of the model. They showed shifts in dominant species of microbiota in HFA mice after administration of human intestinal microbiota by using 16S rRNA gene sequence and terminal restriction fragment length polymorphism analyses. They concluded that the intestinal microbiota of HFA mice and their offspring reflected the composition of individual human intestinal bacteria with some modifications and that the intestinal microbiota of HFA mice represents a limited sample of bacteria from the human source and are selected by unknown interactions between the host and bacteria. They made the point that there is a need to further the establishment of a suitable model for study and to clarify

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Table 1. Summary of conclusions from pivotal papers.

Reference	Study subjects	Analysis	Conclusions
Mallett *et al.* (1987)	Conventional rats, HFA rats and human faeces	Biochemistry	Concluded that flora was similar between all three study subjects on basis of indirect measurements and only true for 3 of 4 tested enzymes
Hirayama *et al.* (1991)	HFA mice	Bacteriology	*Bifidobacterium* not implanted in HFA mice
Hirayama *et al.* (1995)	HFA, conventional mice and human faeces	Bacteriology and Biochemistry	Differences in implanted flora, *Bifidobacterium* not implanted in all mice. Differences in enzyme activities and bacterial metabolism
Hazenberg *et al.* (1981)	HFA, conventional mice and human faeces	Bacteriology	HFA flora similar to man but distinct from conventional mice
Pecquet *et al.* (1986)	HFA mice and human faeces	Bacteriology	Concluded similarity in flora but only screened *Enterobacteriaceae* and total anaerobes

the details of the interaction between the host and the associated bacteria. Indeed, the use of molecular techniques has greatly aided our understanding of the human gastrointestinal flora. Licht and colleagues (2007) showed that while the HFA rat model may have some utility there are indeed differences between the stabilized implanted bacterial flora and that of the donor. In particular, they found that the rat gut environment specifically selected for *Ruminococcus* spp. originating from the human faecal sample, speculating that it was the high content of cellulose fibres in the standard rat feed that provided a selective advantage for these cellulose-degrading species. They speculated if HFA rats were fed with a diet more closely resembling a human diet, the dominating strains in the rat gut may be even more similar to those of the human gut, and that for example the *Ruminococcus* spp. would not have been as efficiently selected in the rats.

The results raise questions about the adequacy of the human faecal microorganisms associated rodent as a definitive model to study the ecology of the human intestinal tract. The disappearances of selected species and failure of others to colonize may influence the outcomes of the studies. Moreover, the greatly reduced metabolic activity of the human faecal microbiota in the mouse may diminish the significance of the results of metabolic studies when extrapolated to the human situation. However, the model might have utility in studies on colonization resistance against various enteric pathogens.

Conclusions

While there have been few studies that have carried out sufficiently detailed direct comparisons of the microbial flora of conventional rodents, HFA rodents and donor human faeces, there are a number of indirect studies that have compared the predictive nature of the HFA rodent and conventional rodent model with that of man. These suggest, on balance, that the HFA rodent model is more likely to be predictive of what occurs in man than will the conventional rodent model. There are of course limitations to the HFA rodent model, which have been highlighted by a number of workers most notably the failure of *Bifidobacterium* spp. (Raibaud *et al.*, 1980; Hirayama *et al.*, 1991) and in some cases *Lactobacillus* spp. (Raibaud *et al.*, 1980) to establish. Whatever stance is taken workers in this area must be mindful of the views of Rogers and Fox (2004), who considered the role of rodent models in the study of infectious gastrointestinal and liver cancer and stated that no meaningful conclusions can be drawn from studies in rodents without knowledge of how the species, strain and gender of the animal may affect experimental outcomes. They argued that results may be highly divergent when the same protocol is applied to different mouse populations, and care must be taken when extrapolating findings from a single inbred mouse to human disease. They made the point that little attention is often given to animal husbandry and the environment when designing and interpreting rodent models of infectious gastrointestinal and liver cancer. Of importance to this paper was their view that animal stress associated with overcrowding, inadequate sanitation and variations in temperature, humidity and light cycles may predispose otherwise resistant animals to adverse disease outcomes and in the case of the enterohepatic system, differences in study outcomes may be attributed to endogenous gut microflora. Microbial population differences may be pronounced even between different rooms and cages within the same facility. As we move forward in our understanding of the gastrointestinal ecosystem, it will be crucial to embrace a systems biology approach involving not only microbiologists but scientists from other disciplines. As Tannock (2008) recently explained, 'The technological approaches to achieve these goals are at the fingertips of microbiologists: metagenomics to access and assess community genetics and metabolomics to analyze functional attributes of the the indigenous microbiota in concert with that of the host'. Clearly, one model will not suffice yet data from models such as the HFA rodent will no doubt be important as we seek to unravel the mysteries of the gastrointestinal tract flora, the implications of that data can only be fully understood if we are aware of the limitations of the model.

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