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Considerations for Choosing Appropriate Animal Models to Study Inflammatory Bowel Disease

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Abstract

This article examines several animal models used to investigate mechanisms involved in the induction and progression of inflammatory bowel disease in people. The use of appropriate animal models to study intestinal inflammation requires careful consideration as each model has strengths and limitations for investigating disease, and no single model provides a complete understanding of the disease process. In as such, it compels researchers to carefully contemplate the advantages and disadvantages of each animal model, and to consider the process of choosing the best animal model(s) as an essential component of the experimental design.

Keywords: animal models, inflammatory bowel disease, intestinal physiology, intestinal immunology, microbiome

1. Introduction

Inflammatory bowel disease (IBD) is a terrible and debilitating disease that affects people worldwide regardless of age, ancestry or socio-economic standing in society. Inflammatory bowel disease is composed of two clinical entities, Crohn's Disease and Ulcerative Colitis and these clinical manifestations of chronic recurring inflammatory disorders of the intestinal mucosa impose a significant impact on human health and healthcare expenditures (Abraham & Cho, 2009; Kaser, Zeissig, & Blumberg, 2010). The economic costs of IBD to health care can range in the billions of dollars annually, and indeed in 2012, the economic costs in Canada were greater than 2.8 billion ($12 000/IBD patient) (Rocchi et al., 2012). Due to the significant ramifications of IBD on people's lives, it is imperative that researchers gain better insight into the pathogenesis of these disorders. This will potentially enable investigators to develop effective mitigation strategies. The underlying cause of IBD is multifactorial and involves an interplay among the host, the intestinal environment (i.e. diet), and the intestinal microbiota. As such, it behooves researchers to employ complex multi-system research models to study the pathogenesis of IBD, and importantly, whole animal models are uniquely capable of investigating physiologic, immunologic and microbiological mechanisms involved in IBD. However, choosing an animal model to investigate IBD can be challenging, and it is important that the characteristics of each model are considered when selecting the best one.

2. Considerations for Choosing the Most Appropriate Animal Model

The various animal models developed for studying IBD and some more common models include: invertebrates (helminths and arthropods), fish, small animals (rodents), larger species (dogs, pigs, ruminants, and others), and non-human primates (NHP). Nematodes and *Drosophila* are used primarily to investigate changes in innate immunity associated with IBD. Nematodes lack an acquired immune response, but similar to people, nematodes have innate intestinal immune responses with analogous signaling pathways, produce antimicrobial peptides, and possess an intestinal microbiota (Lin & Hackam, 2011; Felix & Duveau, 2012). *Drosophila* are insects within the order Dipetera (flies) and used to investigate changes in the host genome and cellular signaling associated with

IBD. *Drosophila* have a NF-$_K$B signaling pathway similar to people and they produce NADPH oxidase dependent antibacterial reactive oxygen species (Bangi, Pitsouli, Rahme, Cagan, & Apidianakis, 2012). Both innate and adaptive immune responses can be assessed in fish. Zebrafish are considered a superior model to investigate IBD in comparison to invertebrates; zebrafish have a more complex microbiota than nematodes and arthropods, and they exhibit similar intestinal cellular and architectural structure to people (Lam, Chua, Gong, Lam, & Sin, 2004). Moreover, fish will develop extra-intestinal lesions associated with intestinal inflammation, similarly to people with IBD. Although fish and invertebrates have the advantages of high fecundity and low husbandry costs, invertebrates in particular are limited in investigating the complex interaction between immune systems (i.e. they lack an acquired immune response) in associated with the development and progression of IBD.

Rodent models are most commonly used for studying IBD. Mice in particular are useful to investigate specific physiologic and immunologic cellular processes involved in IBD, in large part as a result of the large number of engineered genetically backgrounds available (A. Mizoguchi, E. Mizoguchi, & Bhan, 2003; Engelman & Kerr, 2004). Mice share many of the same intestinal specific genes with human beings (Waterston et al., 2002), they possess a comparable intestinal tract in both function and structure, and they exhibit similar intestinal microbial diversity (Dethlefsen, McFall-Ngai, & Relman, 2007; Bryda, 2013). A plethora of engineered mice models have been used to elucidate cytokines involved with innate and acquired intestinal immunity, to examine barrier function, and to understand cell signaling systems. Relative to human beings, the intestinal tract in mice possesses the same intestinal cell types (enterocytes, goblet cells, Paneth cells, enteroendocrine cells, mesenchymal cells and myocytes), and it is similarly organized in histologic structure (Mahoney, Stappenbeck, & Milner, 2008). Mice also possess a diverse bacterial community structure populated with *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* bacterial species similar to people (Waterston et al., 2002). Rats have some of the same attributes as mice, but lack the large repertoire of genetically modified strains. The larger size of the rat, however, facilitates the use of certain experimental techniques, since the larger distal colon allows easier administration of inducers (chemical irritants) of intestinal inflammation (Irving et al., 2014; Nyuki & Pittman, 2015).

Mice and rats share the same advantages as invertebrates and fish models. Rodents have high fecundity, low husbandry costs, and there are many commercially reagents (i.e. monoclonal antibodies) available to study disease processes, particularly for mice (Bryda, 2013). Rodents possess a number of significant disadvantage for studying IBD. Their relatively small size limits the number and type of analyses that can be conducted. Indeed, the small size of rodents often requires 'pooling of tissue' from many experimental animals, and a subsequent increase in the total number of animals required, a situation that is both costly and requires further ethical considerations. Moreover, the small size of rodents limits the use of sophisticated investigative techniques, namely, colonic endoscopy with biopsy collection, radiotelemetry, and ultrasound (Fujimoto, 2003; Helwig, Ward, Blaha, & Leon, 2012).

Large animal species can be used to study IBD. Dogs develop IBD in a similar fashion to human beings and are used to identify intestinal biomarkers of IBD. The canine model has been used to explore the expression of Th1 cytokine profiles in inflamed intestines and to investigate the ability of probiotics to increase expression of anti-inflammatory cytokines and mitigate disease (Jergens et al., 2009; Rossi et al., 2014). Swine models are frequently used as monogastric models as their genetic background, and their intestinal morphology and function are homologous to people. Indeed, the swine genome is closer in size, nucleotide number, and genetic homology to the human genome than either rodents or canines (Walters et al., 2012). The anatomic (stomach to cecum) and histologic structure of the pig intestine as well as the physiologic and immunologic functions of the intestine are similar to humans. Pigs produce similar digestive enzymes, transport and secretory proteins, and their capacity to absorb water and nutrients are comparable with people (Patterson, Lei, & Miller, 2008; Zhang, Widmer, & Tzipori, 2013). Pigs also have high numbers of plasma and intraepithelial γδ T cells, and they are an excellent model to examine the effects γδ T cells on the induction of IBD (Kazen & Adams, 2011). Ruminants are foregut fermenters, and their utility for studying the pathobiology of IBD has not extensively examined. Ruminants are useful, however, for investigating the relationship between specific microorganisms and IBD. For instance, research to study the association between inflammation and *Mycobacterium avium* subsp. *paratuberculosis* has used a ruminant model (Greenstein, 2003). Swine and small ruminants are particularly useful to study IBD as these animals are domesticated and tractable species ideal for establishing novel surgical models to study IBD. Fistulated cannulation of the gastro-intestinal tract in swine and ruminants has been used to collect intestinal digesta in nutritional studies, and could be used to investigate the impact of a number of dependent variables such as diet on IBD (Ivan & Johnson 1981; Jacobson et al., 2001), including the role of the microbiome on

disease. Interestingly, the recently developed recovery 'intestinal loop models' have been used in both sheep (Gerdts et al., 2001; Uwiera, Kastelic, & Inglis, 2009; Inglis, Kastelic, & Uwiera, 2010) and pigs (unpublished data) to determine the effects of microorganisms, activators of immune responses, and inducers of inflammation on both intestine and host health in a highly prescribed manner (e.g. localization of treatments in specific locations in the small intestine). Furthermore, the collection of both intestinal and non-intestinal lymph provides insight to effects of diets, therapeutic agents, and modulators of immune function on immune surveillance and processes involved in the pathogenesis of IBD (Uwiera et al., 2001; R. R. E. Uwiera, Mangat, Kelly, T. C. Uwiera, & Proctor, 2016). Importantly, the use of larger animal models to study IBD facilitates the collection of large quantities of tissue without pooling samples, and thereby reduces the numbers of animals required. There are several disadvantages of larger animal models to investigate IBD. For example, there is a paucity of commercially available reagents to study cellular and immune function within the gut relative to rodent models. The large size of the animals requires more specialized handling facilities, and increased husbandry costs. Swine are genetically heterogeneous relative to rodents, and they have substantially longer gestational, weaning and growth periods, which represent logistic issues with regard to the rapid generation of large numbers of offspring required to study IBD (Knowledge Center, http://www.thepigsite.com).

Non-human primates (NHPs) are considered to be the 'best model' to investigate IBD as their genetic background, physiologic, anatomic, and immunologic similarities to people are undeniable. Non-human primates can develop both spontaneous and experimentally induced intestinal inflammation, and similarly to people with IBD, the development of intestinal inflammation is associated with a Th-1 directed pro-inflammatory cytokine response (Ramesh et al., 2005; Gardner & Luciw, 2008). The structure of bacterial communities in the intestine of NHP species are representative to people (Yildirim et al., 2010). Finally, NHP are used to investigate the impact of intestinal inflammation on the activity of the gut brain- axis, and to explore changes in higher brain functions namely, behavior and anxiety in primates with enterocolitis (Bercik, Collins, & Verdu, 2012). A cautionary note, the use of NHP in IBD research requires careful reflection as using NHPs in animal research remains controversial and ethical justifications for using these animals in research must be beyond reproach. Employing NHP in research can also be cost prohibitive, as housing NHP requires specialized bio-secure facilities, and highly trained animal care personnel and veterinarians to ensure proper animal care (Reinhardt & Roberts, 1997). In addition, NHP can also serve as reservoirs of highly virulent zoonotic pathogens. For example, captive adult macaques often carry *Cercopithecine herpesvirus* (B virus), a virus that is relatively benign to the NHP host, but following transmission to people though body fluids, bites or scratches can induce fatal meningoencephalitis and myelitis (Huff & Barry, 2003).

3. Implications

Choosing the correct animal model to investigate the induction and progression of IBD can be a difficult process. The researcher must not only determine which 'specific mechanisms' associated with IBD need investigation, but also identify the most appropriate animal model for providing reliable data in a cost effective and ethical manner. As such, an understanding of the advantages and disadvantages of each animal model is required to determine the best animal model to study IBD. Choosing an animal model that delivers the broadest amount of knowledge of IBD is ideal. Unfortunately, no single animal model capable of providing a 'complete set of data' on the processes involved in induction and progression of IBD exists. To overcome this challenge, investigators must often employ two or more animal models; using the advantages of each model to deliver the most comprehensive collection of information on processes involved in IBD. A possible experiment with two animal models could include rodents to study cellular processes involved in intestinal inflammation in concert with swine recovery 'intestinal loops' to investigate the effects of inflammation on the intestinal mucosa within different anatomical regions of the gut. Ultimately, the choice of the appropriate animal model(s) needed to investigate mechanisms involved in IBD requires careful consideration by researchers and should be an integral component of the experimental design.

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