Mistakes in mouse models of IBD and how to avoid them

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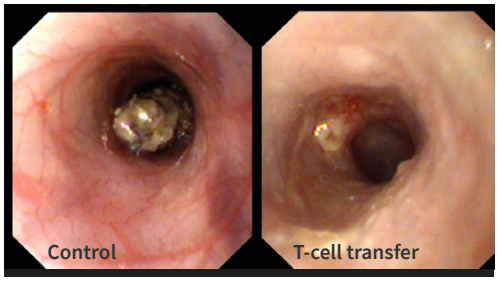

In general, mouse models of colitis are used to study its pathophysiology and for the development of new treatment modalities for inflammatory bowel disease (IBD). For the latter it is essential to select a mouse model that has many overlapping features with human IBD. More than 50 experimental colitis models have been developed and they have provided us with very useful insights into IBD physiology, as reviewed by Bouma and Strober[1] and others,[2–4] but they have limited use in predicting the clinical relevance of therapeutic targets in IBD.[5]

Experimental colitis models broadly fit into four different groups. First is spontaneous colitis, resulting from a naturally occurring genetic abnormality. Second is induced colitis occurring as a consequence of a targeted mutation or the introduction of a transgene. Third is induced colitis resulting from administration of different exogenous causative agents. Fourth is induction of colitis by manipulation of the immune system. We have learned a great deal from these models about the involvement of genetics, the microbiota and the role of different cells and the mucus layer in the development of IBD.

Here we discuss the major mistakes that are made using experimental colitis models, based on our own experience and the scientific literature. Recently increased awareness has developed for the necessity to improve the methodological quality of animal studies.

Mistake 1 Choosing an inadequate or obsolete model

Choosing the right mouse model is a major issue in studies of experimental colitis. In previous years some quite extensive overviews of the different models available have been published,[3,6–8] but most authors refrain from giving advice on the best model to choose. Indeed, if any advice is given it is very limited. For example, Goyal et al.[7] concluded that the "… currently available animal models are relevant to human IBD if they are chosen carefully (chronic, immune mediated)" and DeVoss and Diehl[6] indicated that a successful approach requires "… careful utilization of pathway models to query specific scientific or efficacy questions."

There are a number of chemically induced acute colitis models that are easy to use, rapid and of low cost and therefore widely used. However, these models may not be the best models to study IBD, because chemical damage to the gut epithelium results in self-limiting inflammation rather than chronic inflammation. Comparative analysis of colonic gene expression in the 2,4,6-trinitrobenzene sulfonic acid (TNBS), dextran sulfate sodium (DSS) and T-cell transfer models with human IBD revealed that the pattern of gene expression in the T-cell transfer model most closely reflects altered gene expression in IBD.[9] Chemically induced models should only be used if the intention is to study the physiology of epithelial regeneration or intestinal wound healing.

In general, the different mouse models of colitis may reflect human IBD subtypes as described in table 1. However, there is not one single experimental colitis model that resembles all aspects of human IBD. Making the choice of which model to use should combine the research question and the IBD subtype to achieve the best outcome.

Mistake 2 Not using standard protocols for induction of colitis consistently

In 2006, a critical appraisal of experimental colitis induction using TNBS revealed that the protocol followed differed in each of the studies included.[10] Indeed, the mouse strains used, mouse age, dosing and times of TNBS administration, percentage of ethanol used and the duration of the experiment all varied. In 2007 Wirtz et al.[11] published experimental protocols for the chemical induction of colitis in mouse models, thereby setting the gold standard for this methodology. Unfortunately, since then not many studies using these models seem to have followed the procedure described in this protocol.[5] For the T-cell transfer model an excellent protocol, including critical para meters and troubleshooting, has been published in Current Protocols in Immunology[12] and by Ostanin et al.[13]

The lack of consistency in the experimental protocols used for the chemical induction of colitis in mouse models hampers reproducibility, which is fundamental for any scientific experiment.[14,15] In addition, standardization of environmental factors, such as circadian rhythms, nutrition, age, sex and strain are important confounders that have to be identified and acknowledged.[16,17] To ensure consistency and reproducibility, the same protocol and environmental circumstances should be secured in every experiment and preferably shared by several laboratories.

Mistake 3 Failing to randomly allocate animals to their experimental group

Randomly allocating animals to groups is a relevant issue when studying intestinal inflammation, because it ensures that subtle differences between the animals are unlikely to influence the experimental outcome. Usually, the body weight (or body weight change) of the animal, besides sex and age, is the most important parameter to account for in the randomization process. As the composition of the microbiome has a great influence on the development of intestinal inflammation,[18] and this can differ between cages, the animals in each experimental group should be co-housed, so that representatives of the different experimental groups are within a single cage, and the groups are replicated across a series of cages.[19] This way the differences between the groups and the cages can be measured independently.

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Model	IBD subtype
Acute DSS colitis	Acute self-limiting colitis, focus on innate immunity, ulcerative colitis like
Acute TNBS colitis*	Acute self-limiting colitis, focus on NF-κB activation, no model for IBD
Chronic DSS colitis (cycles of DSS)	Chronic progressive inflammation, mix of innate and adaptive immunity, ulcerative colitis like
Chronic DSS colitis (recovery phase)	Acute inflammation followed by recovery with low-grade inflammation, mix of innate and adaptive immunity, ulcerative colitis like
Established TNBS*	Acute inflammation, mix of innate and adaptive immunity, DTH reaction, Crohn's disease like
$CD4^{+}CD45RB^{high}$ transfer	Chronic progressive inflammation, focus on adaptive immunity, Crohn's disease like
IL-10$^{-/-}$	IL-10(R)-deficient patients
Various transgenic models**	Colitis, ileitis, Crohn's disease and ulcerative colitis like properties

Table 1 | Overview of experimental colitis models and the related IBD subtypes. An extensive list of experimental IBD models and their different IBD phenotypes has been published elsewhere.[51] DSS; dextran sulfate sodium; DTH, delayed-type hypersensitivity; TNBS, 2,4,6-trinitrobenzenesulfonic acid. *For TNBS models, see ref. 10. **Reviewed in ref. 52.

In some cases it may be impossible to mix the experimental groups in one cage. For example, mice may not be mixed due to gender differences. Also, it is impossible to mix DSS animals and control animals (i.e. non-DSS-treated mice) in one cage. If this is the case it is recommended to randomly allocate the cages within the same room. In addition, when the animals are sacrificed the sequence should be randomized to avoid the introduction of bias. There are several ways to randomly allocate the animals used in an experiment. A random sequence generator for randomization of animals and the random integer set generator for randomization of an intervention can be found at www.random.org.

Mistake 4 Not blinding the study

Another important consideration when setting up an accurate animal experiment is that it should be blinded at several levels. The division between experimental and control groups should be blinded to avoid selection bias. The person who is responsible for the daily care of the animals should be unaware of the intervention(s) to avoid performance bias. Moreover, the people involved in determining the outcome para meters should not be aware of the intervention(s) to avoid detection bias. In general, blinding can be realised by having an independent outsider give each animal an individual mark/number coupled to the intervention(s) and only disclosing the mark/number at the end of the experiment. In general, the same care and quality control should be incorporated in animal experimentation as is customary in human clinical trials. Hooijmans et al. recently described a tool that can be used to assess the risk of bias for animal studies.[20]

Mistake 5 Inadequate use of outcome parameters

As with research in human patients, one problem when using animal models is deciding what the most important disease parameter/primary endpoint is in fundamental and/or translational studies. Semi-quantitative evaluation of intestinal histo(patho)logy is considered to be the gold standard in animal models of intestinal inflammation. However, these histology-based scoring systems are not uniformly used in the literature. Most of these scoring systems include different sub-scores of histological aberrancies that are present in the animal model, such as crypt loss or immune cell infiltration.

For different models different sub-parameters are relevant, for example epithelial destruction in the DSS model, or epithelial hyperplasia in the T-cell transfer model.[21] Therefore the most reliable scoring system for the model should be chosen. The slides should be blinded comprehensively without any reference to an individual animal or experimental group. As histopathological scores are given as an ordinal read-out (i.e. 0,1,2…) the median value is the most appropriate measure for central tendency within groups. This hampers the calculation of the number of animals needed per group, as the mean is used to calculate group sizes.[22]

Mistake 6 Insufficient matching of control animals

When transgenic or knockout animals are used to study the effect of the transgenic/knockout genes wild-type animals are often used for comparison. As the microbiome has a great influence on the experimental outcome in these mouse models of IBD this has to be taken into account.[18,23–25] There is good evidence that the composition of the microbiota differs in animals that are not co-housed. Jacobsson et al. observed that two C57BL/6 mice colonies maintained in different rooms at the same facility had a different gut microbiota.[26] In addition, Ivanov et al. found that C57BL/6 mice obtained from different commercial vendors displayed differences in the numbers of Th17 T cells that could be related to the presence of specific bacterial taxa.[27] This difference in microbiota was recently confirmed in another study for additional strains of mice.[28] Another aspect that has to be taken in consideration is that certain drugs can have an effect on the microbiota composition and in this way affect disease development.

When using wild-type animals as controls in experiments with genetically modified mice, litter-mate wild-type animals should be used. As genetic modification can result in an altered microbiota,[29] and this can be transferred to co-housed control mice together with increased susceptibility to colitis, the use of co-housing to ensure similar microbial composition should be done with precaution. To avoid the possible bias introduced by the microbial composition, models with specific microbiota can be used. Regardless, in the future it may be obligatory to characterize the microbiota in every study and incorporate this information into data evaluation.[23]

Mistake 7 Not being aware of the susceptibility differences of the available mouse strains

One of the insights in IBD physiology reviewed by Bouma and Strober[1] is that the host genetic background determines susceptibility to colitis. Various studies have described that the differences in susceptibility to chemically induced colitis is strain dependent.

The C3H/HeJ, C3H/HeJBir30 and C57BL/6 strains are highly susceptible to DSS-induced acute colitis, while BALB/c mice only develop colitis when higher percentages of DSS are administered.[31] Also, the recovery phase of the disease after 5 days of administering DSS differs between C57BL/6 and BALB/c mice—C57BL/6 mice develop a severe chronic inflammation, whereas BALB/c mice resolve the colitis after the acute phase.[31]

In TNBS colitis the difference in susceptibility to colitis between SJL/J (susceptible) and C57BL/6 (resistant) mice is associated with the ability to mount an IL-12 response to lipopoly-saccharide (LPS).[32,33] IL-12 is the major cytokine for the differentiation of Th1-CD^{4+} T cells. For the mouse models in which T cells play a role it is important to realize that, in general, mice with a C57BL/6 background are more prone to develop a Th1 response, whereas BALB/c mice have a tendency to develop a Th2 response[34] when exposed to pathogens.

In the T-cell transfer model mice both C57BL/613 and BALB/c35 backgrounds are used. In IL-10 knockout mice severe intestinal lesions develop in mice with a 129SvEv or BALB/c background, while C57BL/6 strains are relatively resistant to the development of colitis.[36,37] In C57BL/6 mice colitis induction can be accelerated by peroral administration of piroxicam, a nonselective nonsteroidal anti-inflammatory drug (NSAID).[38]

To avoid the differences in susceptibility introduced by these extreme phenotypes, it might be an option to introduce the use of a collaborative cross-mouse genetic reference population as a new less biased resource in IBD research.[39,40]

Mistake 8 Not being aware of the differences in disease susceptibility between the sexes

For most autoimmune diseases there is a clear difference in susceptibility between the sexes, with females more frequently affected than males.[41] In experimental models of colitis sex-specific effects have also been described. For DSS colitis greater male susceptibility has been observed,[30,42] and for TNBS the wasting disease has been shown to have a greater effect on female mice.[33]

Most experiments are performed with either male or female mice. However, in incidental experiments in which both sexes have been used,[43–45] or a comparison was made between experiments,[5] differences can be observed. In the T-cell transfer model both male[13] and female[35] mice are used. In general, DeVoss et al.[6] recommend using female animals if possible. They indicate that male animals are more prone to display aggressive behaviour resulting in fighting, with the resulting stress and wounds potentially having a negative impact on a study. This finding hampers the random allocation of the mice because non-littermates cannot be housed together. However, single housing of male animals also has an effect on wellbeing[46] and is expensive. In a study in which several aspects of the current usage of experimental colitis models was analysed, the predominant use of male animals was observed.[5]

Mistake 9 Poor reporting quality

Experimental colitis models are frequently used to try to answer several biomedical research questions in IBD research. For successful translation of the knowledge from these studies to the clinic they should be well designed and reported, which does not seem to be the case.[5,47]

Quality assessment of animal experiments includes several different features and questions, and should at least include the items discussed previously. Is the research question specified and clear? Are animals randomly allocated across groups and is the outcome assessment randomly allocated across groups? Are the group characteristics clearly described and do they use a correct control group? Do they use a blinded outcome assessment? Is the timing clear? Which scoring system is used for histology? Are the treatment protocols clearly described? Are the number of animals per group clear and what is reported about the animals excluded from analysis? If mentioned, is it clear what the exclusion criteria are? Do the authors report complete outcome data?

Several tools are available to improve the reporting of outcomes in experimental colitis models. With the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, which consists of a 20-item checklist, the reporting quality of all specific characteristics of the animals (including species, strain, sex, age, genetic background), housing details and methodology will be boosted. Encouragingly, more and more editors of scientific journals have adopted these guidelines and urge authors of submitted papers to use them.[48] In addition, Bramhall et al. have published a checklist of essential and desirable criteria specified for reporting in animal models of colitis.[47]

Mistake 10 Inadequate administration of therapeutic agents

Experimental colitis models are frequently used for preclinical drug evaluation. The pharmacological approach is an important topic, and several aspects and considerations have been reviewed by Koboziev et al.[49] Here, we focus on some of the main issues.

Of great importance is being aware that in chemical models of colitis the administered compound can potentially interfere with the DSS or TNBS and result in a reduced colitis induction. Also, in DSS colitis it must be confirmed that the treatment regimen does not influence the water consumption. So, this should be carefully monitored. Drugs can also have an effect on the microbiota composition and in this way affect disease development.

In experimental colitis models in which the induction of the colitis is fixed on a specific time point, as is the case in the chemically induced models, it is calculated that 78% of the treatments are applied before or within 24 h after the induction of colitis.[5] In this situation it can be questioned whether a positive effect is due to actual treatment or interference with induction of colitis. It is actually essential to treat established disease. Koboziev et al[49] indicate that "…one of the best predictors of clinical efficacy of a drug is its ability to reverse established disease in at least two different animal models of chronic intestinal inflammation." This idea is also advocated in a recent commentary on reproducibility.[17] With the introduction of endoscopy,[50] researchers are able to investigate the effect on established disease more efficiently, enabling the comparison of disease characteristics (semi-quantitative score of endoscopy) before and after treatment for each individual animal (and do paired statistical analysis).

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