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Important Lessons Derived From Animal Models of Celiac Disease

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Abstract

Several animal models have been recently developed to recapitulate various components of the complex process that is celiac disease. In addition to the increasing diversity of murine models there are now monkey models of celiac disease. Mouse strains are just beginning to address the complex interactions amongst the innate and adaptive immune responses to gluten, as well as gluten dependent autoimmunity in celiac disease. The most important conclusion that these models have provided us with is that while all three components (innate gluten sensitivity, adaptive gluten sensitivity, and autoimmunity) are independent phenomena, all are necessary for celiac disease to develop.

Although the first modern description of celiac disease was made over 100 years ago by Samuel Gee in 1888, most of our understanding of the disease comes from studies on humans. It is only been in the last 10 years that significant strides towards generating robust and useful animal models have been made [1]. These advances include the generation of animal models of the three main components of celiac disease: the HLA association (DQ2, DQ8), the predisposition towards autoimmunity, and the innate immune responses to gluten. This review will evaluate the models and then discuss what conclusions can be drawn from them about the pathogenic role of each of the three components of celiac disease.

Dogs

The initial animal model of celiac disease was the Irish setter, as studies done in the 80's determined that when the Irish setter was administered a wheat containing diet as pups, they would develop partial villous atrophy and intraepithelial lymphocyte infiltration [2–7]. However, this transient gluten dependent enteropathy observed in the Irish setter was determined in later studies to be independent of the dog MHC II, and was therefore not a CD4+ T cell mediated disease of gluten sensitivity, though the more recent emphasis on innate response to gluten may again make that model relevant [8].

Monkeys

Another “spontaneous” animal model of celiac disease is that of the rhesus macaque and has many features of celiac disease [9]. In this model, gluten sensitive rhesus macaques on a

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Declaration of Interest

The authors declare that there is no conflict of interest.

gluten containing diet develop villous atrophy as well as anti gliadin IgA and IgG [9]. Antibodies against tTG, both IgG and IgA were shown in a later publication to be elevated in some animals but these levels did not change with the removal or the re-introduction of dietary gluten [10]. Currently, the incidence is 1:125 for macaques that have elevated anti tTG and anti gliadin IgA levels, which is similar to the incidence of celiac disease in humans in the US population but very low for an animal model [10]. Also, it has not yet been determined as to whether this phenomenon is associated with MHC II [10].

However, this model is good for testing the applicability of different novel therapies of celiac disease such as the barley endoprotease, EP-B2 [11]. Another significant value of this model is the availability of healthy controls. As such, this model has been used to address the transepithelial transport of a peptide that is immunogenic for celiac disease, called the “33-mer” for its unique property of being 33 amino acids in length [10]. This peptide crossed the epithelial barrier only in the monkeys that displayed celiac-like enteropathy, but not in the healthy controls. Also, intestinal permeability and disruption of intercellular tight junctions was only found with the gluten sensitive monkeys on a gluten containing diet, suggesting that gliadin-induced intestinal permeability is an aberrant response to gliadin and may be a requirement for the later development of gliadin-induced enteropathy.

Rats

Germ free Wistar AVN rats were used to study the effects of administering gliadin immediately after birth until 63 days old [12]. This led to the shortening of villi, crypt hyperplasia, and an increased number of intestinal CD8⁺ TCR $\alpha\beta$ ⁺ and CD4⁺ TCR $\alpha\beta$ ⁺ lymphocytes. Further supporting the achievement of gluten sensitivity, transfer of intraepithelial lymphocytes (IELs) from the gliadin treated rats to the intestinal loops of untreated rats produced pathology in the naive recipient, demonstrating that the IEL compartment contributed to the development of enteropathy. Further extending this work, the same group determined that commensal bacteria affected the ability of gliadin to induce intestinal permeability [13]. Specifically, they found that the administration of gliadin and IFN γ with *B. bifidum* to intestinal loops resulted in only small amounts of gliadin in the lamina propria (translocation), but with the administration of Shigella with gliadin and IFN γ , they found large amounts of gliadin in the lamina propria. This study demonstrated that the composition of the commensal bacteria affected the level of intestinal permeabilization induced by gliadin in an MHC II-independent fashion.

Non-transgenic Mice

Mice are more feasible as models of celiac disease, given their shorter lifespan, and advances in the genetic manipulation of mice. Studies done with the in-bred line of Non-Obese Diabetic (NOD) mice demonstrated that they have a spontaneous sensitivity to gluten that does not require parenteral sensitization. When maintained on standard wheat containing chow, NOD mice have a significant increase in the numbers of CD3⁺ IELs in the small intestine (21.5 ± 7.2 Standard Chow vs 12.8 ± 6.2 Gluten Free Chow), as well as a subtle decrease in villous height [14]. Also, the level of epithelial expression of mouse MHC II molecules was increased while on a gluten containing chow [14, 15]. However the antibody response to TTG, that is clearly gluten dependent in celiac disease, was more related to the NOD strain and not gluten dependent [15].

Genetically Engineered Mice

In another mouse model of celiac disease, a cellular transfer method was used that was based on the transfer of T cells enriched for gliadin responsive effector/memory T cells [16] to recipient mice that lacked B and T cells (Rag 1^{-/-}). In this model, CD4⁺ CD45RB^{lo}

CD25-cells were isolated from C57BL/6 mice that were sensitized to gliadin by injections of gliadin at the base of the tail and subsequently transferred to Rag1^{-/-} mice on a gluten containing chow. The recipient Rag1^{-/-} mice on a gluten containing chow developed histological features similar to those of celiac disease. These included lymphocytic infiltration of the villi and lamina propria, crypt hyperplasia, and villous atrophy. There were also increased levels of IFN γ and IL17 transcripts in the duodenum of the recipient mice. In contrast to the previous animal models, this one was dependent upon MHC II; however, this was murine MHC II and not DQ2 or DQ8 (one of the two alleles associated with celiac disease) and thus, this model can be improved with the use of HLA transgenic mice.

Initial work with HLA transgenic mice was done with mice that express HLA-DQ8, one of the two DQ alleles that are associated with celiac disease [17]. In this study, DQ8 transgenic mice were sensitized to gluten and their T cell response and antibody production evaluated [18]. Although they did not develop symptoms, they did develop a strong T cell proliferative response as well as antibody response with increased levels of anti gliadin IgG [18]. A later study demonstrated that after intraperitoneal injection of gliadin and 3 weeks of gavage with supplementary gliadin these mice had increased numbers of CD3⁺ intraepithelial lymphocytes (IELs) as well as cholinergic dysfunction resulting in altered muscle contraction and ion transport [19]. These DQ8 transgenic mice were also used to demonstrate that the TCR repertoire induced by injection with native forms (not deamidated) of DQ8 binding gliadin peptides had a heteroclitic (stronger) response to the deamidated forms of the peptides [20]. This work suggests that the following progression would occur. Initially, an aberrant innate immune response to gliadin would activate tTG for the purpose of healing. Then the activated tTG would result in the presence of both native and deamidated forms of gliadin derived peptides. The presence of both forms would then accelerate the T cell response to gliadin, causing further damage, and enteropathy. This heteroclitic response would also explain why most adult celiac patients have a strong T cell response against the deamidated forms of specific gliadin-derived peptides whereas children may retain a strong response to native peptides.

In the DQ8 transgenic mice, specific perturbations to the innate immune system resulted in increased numbers of IELs and the production of inflammatory cytokines such as IFN γ and IL12p70. The first perturbation was the systemic overexpression of IL-15 as driven by the MHC I promoter, D^d [21]. This mimicked the increased levels of IL-15 in the lamina propria of celiac patients [22] and resulted in the expansion of natural killer (NK) and CD8⁺ T cells [21]. The second perturbation was the addition of retinoic acid (a vitamin A metabolite), which promoted the development of an inflammatory (Th1) response against dietary proteins in the IL15 over-expressing DQ8 transgenic mice [23].

In transgenic mice that express both HLA-DQ2 and HLA-DQ8 [24, 25], sensitization to gliadin resulted in a strong T cell response to gliadin, but no overt enteropathy. One group went further and generated a DQ2DQ8 mouse that expressed a gliadin specific TCR that had been identified in the gliadin sensitization studies [26]. The resultant (DQ2DQ8-TCR) mice developed a substantially strong T cell response towards gliadin after sensitization; surprisingly though, these mice did not develop shortening of the villi. This latter result would support the theory that strong T cell dependent responses to gliadin and the development of enteropathy as characterized by the shortening of villi are two phenomena that can occur independently of each other.

Although the IL-15 over-expressing DQ8 transgenic mouse study had incorporated elements of innate immunity (IL-15) together with the adaptive arm of the immune response in celiac disease (DQ8 transgenic), the third element of celiac disease, that of autoimmunity, had not been addressed in the context of the DQ8 transgenic mice. To address the role of

autoimmunity in the development of celiac disease, we used mice that expressed the DQ8 transgene on an NOD congenic background in the absence of endogenous MHC II [27]. In contrast to previous studies though, sensitization to gluten did result in these mice developing a symptom of gluten sensitivity, the skin manifestation of celiac disease, Dermatitis Herpetiformis [27]. This model had many of the elements of dermatitis herpetiformis, including IgA deposits at the dermal epidermal junction, infiltration of neutrophils into the dermis of the lesional tissue, and most importantly resolution of the disease with a gluten free diet and/or dapsone. However, these mice did not develop overt flattening of the villi.

Interaction Between the Intestinal Microflora and Gluten

One powerful use of the mouse models is the ability to radically alter the intestinal microbial ecology through the administration of probiotics, pathogens, and even genetically altered organisms, as well as housing in a gnotobiotic facility, which would eliminate all microflora [28]. All of these approaches have been used to study the impact of the intestinal microflora upon the development of gluten sensitivity, which has recently emerged as a potential contributing factor in celiac disease [29]. It is well known that diet can affect the composition of bacterial species in the intestinal microflora. Switching humanized gnotobiotic mice from a low-fat, plant polysaccharide-rich diet to a Western diet (high-fat/high-sugar) significantly changed the composition of the transplanted human microbiome with notable increases in the members of the Erysipelotrichi and Bacilli classes of the Firmicutes [28]. One study found that *E. coli* and *Staphylococcus* were significantly more abundant in the duodenum of untreated pediatric celiac patients but that this returned to normal after the administration of a gluten free diet [29].

Another study addressed whether the composition of the commensal microflora was changed in DQ8 transgenic mice with the administration of dietary gluten with or without indomethacin [30]. They found that *E. coli* and *E. rectale*-*Clostridium* groups were significantly decreased in gluten-sensitized mice, and that all groups of bacteria evaluated (*Prevotella*, *E. coli*, *Clostridium* *Leptum*, *E. rectale*, and *clostridium* *histolyticum*) were significantly decreased with the administration of both indomethacin and gliadin to the DQ8 transgenic mice. It remains to be seen if any bacterial species increase with dietary gluten in the DQ8 transgenic mice as was found in the study on celiac patients by Collado et al [29].

Testing of Novel Therapies Using Animal Models

As mentioned above, animal models have been used both for proof of concept as well as for exploratory evaluations of the safety, efficacy, and dosing of novel therapies for celiac disease. For such studies, it is important that the animal model replicate as closely as possible the specific human circumstances in which the putative therapy will work. For example, a similar digestive tract would be needed for testing therapies applied to the gut lumen. These therapies include endopeptidases that would inactivate immunogenic gluten peptides. Another study generated polymeric molecules that would bind to gliadin and block the expansion of gliadin specific T cells. One example of this would be the use of the gluten sensitive rhesus macaque model to study the efficacy of a barley endoprotease, called EP-B2. Administration of EP-B2 to a gluten sensitive monkey during gluten challenge inhibited the development of symptoms; however it did have an increase in anti gliadin IgG and IgA and anti tTG IgG, even over the levels of other gluten-sensitive monkeys that had been regularly maintained on a gluten containing diet [11]. This increase in antibody levels with the administration of EP-B2 with gluten would indicate that the T cell response against gluten was diminished with EP-B2, but for unclear reasons, the B cell response was increased. This may be a cause for concern unless such approaches are highly effective at

degrading immunostimulatory peptides. Another strategy that targeted the intestinal lumen used polymers to bind to gliadin. With the use of DQ8 transgenic mice, it was determined that the polymers not only inhibited the formation of immunogenic gliadin peptides, but also inhibited the ability of gliadin to increase intestinal permeability [31]. Correspondingly, there was no increase in the number of IELs and blunted the increase of ion transport seen in the mice treated with gliadin alone.

Tolerance induction

Different approaches have been used to generate tolerance to gliadin in HLA transgenic mouse models. The first approach used intranasal administration of recombinant α -gliadin to DQ8 transgenic mice [32]. This approach attenuated the T cell proliferative response as well as decreased the production of IFN γ after peripheral sensitization to α -gliadin.

A second approach used bioengineered probiotics to suppress inflammatory T cell responses to gliadin in NOD DQ8 transgenic mice [33]. In this study, *Lactococcus lactis* bacteria were engineered to secrete the dominant DQ8 restricted deamidated gliadin peptide, previously identified as immunogenic in DQ8+ celiac patients [34]. When these bacteria were administered to the HLA-DQ8 transgenic mice after parenteral sensitization to the peptide, the subsequent T cell response was greatly diminished. The diminished T cell response was evaluated using a delayed-type hypersensitivity (DTH) response as well as T-cell proliferative response in the sensitized mice. There was also a corresponding increase in FOXP3 positive T cells in the gut associated lymphoid tissue with increases in the production of IL-10 and decreases in the production of IFN γ with the administration of the gliadin secreting bacteria [33].

A third approach to induce systemic tolerance was through repeated intradermal injection of immunodominant peptides. In this study, this was achieved with gliadin derived peptides using HLA transgenic mice that express DQ2 and DR3. This resulted in a suppressed CD4+ T-cell response to gliadin as evaluated by proliferation as well as the production of IL-2 and IFN γ [35].

Important Lessons Learned from the Animal Models

Modeling pathogenesis

The most important lesson that the different animal models of celiac disease has provided us is that celiac disease is a composite of three different phenomena that can each occur independently of each other. The first component is that of an aberrant innate immune response to dietary gluten and/or dietary proteins in general. As was concluded from the work with the IL-15 overexpressing DQ8 mouse, increased levels of IL-15, as found in celiac patients, perturbs the intestinal homeostasis and allows (drives?) the inflammatory T cell response to gliadin as characterized by a strong production of IFN γ . However, overexpression of IL-15 alone does not in itself cause overt flattening of villi.

Another aberrant stimulation of the innate immune system by gliadin that is only found in celiac patients is that of gliadin-driven intestinal permeability. Currently, it is thought that gliadin will bind to CXCR3, which is expressed by intestinal epithelial cells [36]. The crosslinking of CXCR3 results in the release of zonulin (pre-haptoglobin-2), which causes the opening of the tight junctions between the epithelial cells [36, 37]. The rhesus macaque monkey model has shown that gliadin consumption does not result in intestinal permeability in the gluten-tolerant (healthy) monkeys; they only observed this occur in the gluten-sensitive monkeys [10]. Also, their detection of a radio-isotope labeled 33mer (^{125}I -33-mer) in the sera of the gluten-sensitive macaque within 60 minutes after administration demonstrated that the trans-epithelial transport is extremely fast.

Both the transcellular and para-cellular pathways were observed to occur in the gluten sensitive rhesus macaques [10]. In the study that addressed transcellular translocation, fluorescently labeled 33mer was instilled into the duodenum of two healthy (gluten-tolerant) and two gluten sensitive monkeys. Pinch biopsies were extracted 40 minutes after instillation. Staining showed that the 33mer was present in the lamina propria as well as the epithelium, specifically inside individual epithelial cells in “Goblet cell-like cavities”. This result would indicate that both the paracellular and transcellular pathways are occurring in the macaque model. Neither of the healthy monkeys had any 33mer present below the brush border membrane. This result with the healthy monkeys demonstrates that both translocation pathways only occur in the gluten-sensitive monkeys.

The second component of celiac disease would be a strong adaptive CD4+ TCR $\alpha\beta$ + immune response to gliadin restricted by DQ2 and/or DQ8. Many studies using cell lines derived from intestinal biopsies from celiac patients have shown that these cells are the source of the elevated levels of IFN γ in the intestines of active celiac patients. However, as the afore-mentioned studies with animal models show, especially the HLA transgenic mouse models, an exceptionally strong CD4+ T cell response to gliadin does not result in gluten-dependent enteropathy.

In the Irish setter model, there was gluten dependent subtotal villous atrophy similar to celiac disease, but independent of MHC II [8, 38]. Some gluten sensitive macaques developed villous flattening in a gluten dependent fashion; however, it is unknown if this feature is MHC II dependent [10]. This would suggest that villous flattening, a characteristic feature of celiac disease, is disassociated from MHC II.

Genetic studies demonstrate that MHC II molecules only contribute 40% of the risk for developing celiac disease [39]. Therefore, other elements must be contributing to the villous flattening. One such element may be a strong anti tTG production in response to gluten consumption. This autoimmune response against a self-protein characteristic of celiac disease is used as a diagnostic tool and is elevated in celiac patients who have gluten dependent enteropathy [40]. It may be that this antibody plays a heretofore unappreciated pathogenic role in the development of autoimmunity, specifically that of gluten dependent villous atrophy and flattening. Indeed, this antibody has been found to be produced in the intestines of celiac patients as well as deposited at the epithelial membrane of the small intestine [41–43].

Overall then, the animal models have provided us with a new paradigm for the pathogenesis of celiac disease. This paradigm, as depicted in figure one, would consist of three components: an aberrant innate immune response to gliadin that occurs in the context of HLA DQ2/DQ8 as well as perturbations to the regulatory arm of the immune system, resulting in autoimmunity.

Triggers of Celiac Disease

It is likely that all three arms have to be activated simultaneously in order for the flattening of the villi to occur in a gluten dependent fashion as well as a self-propagating fashion. This activation could result as a consequence of environmental factors or behavioral factors. One study showed repeated rotavirus infections during infancy may increase the risk of developing celiac disease [44]. Another study determined that a subset of antibodies against tissue transglutaminase also binds to VP-7, a rotavirus protein [45].

With respect to behavior affecting the development of celiac disease, a number of studies have shown that the introduction of gluten too early in an infant’s diet increases the risk for

developing celiac disease [46]. It has also been shown that breastfeeding can delay the onset of celiac disease [47, 48].

As the current animal models of celiac disease have not yet incorporated these risk factors, it may be beneficial to do so. This would allow us to better understand the specific immunological events that are occurring in the immature intestinal immune system during the initial exposure to gluten. Such analyses could be performed in the different mouse models, and may lead to the overt flattening of the villi, which is a characteristic feature of celiac disease.

Another approach to make the models more powerful would be to increase the complexity through the use of more transgenes. For example, the trafficking patterns and fates of different cell types could be evaluated, as has been successfully done using FoxP3-GFP reporter mice and the cellular transfer model of IBD [49]. Evaluating the roles of cytokines in the triggering, maintenance, and suppression of cytokines could be achieved through using knockout constructs or overexpression constructs as was done with the IL-15 overexpressing DQ8 mouse. These increasingly complex mouse strains will require significant financial investments in breeding programs; however, similar approaches with mouse models of IBD have revealed fruitful insights into its pathogenesis. As more and more countries recognize celiac disease as a growing health concern of national importance, the generation of increasingly complex animal models will not be far behind.

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Figure 1.
The three aberrant responses to gluten necessary for celiac disease.