Controversial view of a genetically altered mouse model of focal retinal degeneration

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Introduction

Recently, Tuo et al. (2012) used a Ccl2–/– Cx3cr1–/– double knockout (DKO) on C57BL/6N genetic background to show tumor necrosis factor-inducible gene 6 recombinant protein (TSG-6) arrest of focal retinal lesions. The DKO strain was generated as a model for focal retinal degeneration after report of senescent Ccl2 single knockout mice developing key characteristics of human age-related macular degeneration (AMD) and the discovery of an association between CX3CR1 single nucleotide polymorphisms and AMD. The Ccl2 and Cx3cr1 deficient DKO strain exhibits AMD-like features such as retinal pigment epithelium (RPE) alteration, immune activation and A2E elevation and thus has been suggested as an appropriate model for human AMD. As compared with independently-generated Ccl2 and Cx3cr1 single knockout strains, DKO shows earlier onset, higher penetrance and more lesions (Fig. 1). Therefore, this strain has served as a valuable tool for screening therapies for human AMD. The recent finding of the accidently introduced crumbs-like 1 (Crb1) mutation and Crb1 rd8-associated retinal dystrophy in the C57BL/6N background has challenged DKO as an AMD mouse model. CRB1 is thought to be involved in maintaining the integrity of the external limiting membrane and mutations in CRB1 result in genetic retinal disorders such as retinitis pigmentosa and Leber congenital amaurosis in humans. The Crb1 mutation results in irregular retinal lesions in the inferior nasal quadrant of the fundus and thus is suggested to have potential implications for all ocular vision research models on C57BL/6N background, including DKO (hereon referred to as DKO rd8).

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While differences in mouse and human physiology limit the formation of drusen proper, infrequent appearance of drusen-like lesions in DKO rd8, which has not been reported in Crb1 rd8 mice, demonstrate distinct retinal pathology between the two strains. In general, C57BL/6N mice seem to have much less severe retinal phenotypes with exception of the foci of mild retinal dystrophy (Fig. 2B) compared with the Crb1 rd8 mice (Bo Chang, personal communication). In addition to photoreceptor degeneration, RPE alteration is a cardinal feature of human AMD. One of the prominent histopathologies in DKO rd8 is abnormal RPE cells, which display hypopigmentation, deapsulation, vacuolation and findings suggest the primary pathology of Crb1 rd8 mice to be developmental defects in Müller cells and photoreceptor inner and outer segment shortening, resulting in the secondary pathology of retinal dysplasia and degeneration of focal photoreceptors. Thus, Crb1 rd8 is a model of retinal dystrophy resulting from malformed Müller cell apical villi. DKO rd8 mice also show photoreceptor pathology (Fig. 2D). However, in addition to photoreceptor dystrophic lesions, RPE degeneration appears to be a featured pathological event in DKO rd8. Whereas retinal folds and pseudorosettes are frequently seen in Crb1 rd8 mice (Fig. 2C), these retinal lesions are seldom found in DKO rd8 (Fig. 2D).
Elevated Lipofuscin Biochemical Marker A2E in DKO rd8

Figure 2. Photomicrographs of C57BL/6J, C57BL/6N, Crb1rd8 and DKO rd8 retina. (A) C57BL/6J strain that does not carry the rd8 mutation shows normal ocular architecture. (B) The C57BL/6N strain displays small rd8-associated dystrophic lesions showing migration of the ONL into the OPL (arrows), preserved photoreceptor IS/OS, and intact RPE. (C) Crb1rd8 displays pseudovesicles and retinal folds in addition to retinal dystrophy involving the inner and ONL (arrows) and thinning of IS/OS (arrowhead). (D) In addition to the rd8-associated lesions observed in C57BL/6N, DKO rd8 shows severe ONL disorganization (arrows), focal loss of IS/OS, and hypopigmentation and vacuolation of RPE (arrowheads). IPL, innerplexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS/OS, inner segments and outer segments; RPE, retinal pigmented epithelium. (hematoxylin and eosin, original magnification, 200×).

Figure 3. Quantification of A2E in C57BL/6J, C57BL/6N, Crb1rd8 and DKO rd8 strains at 2 mo. DKO rd8 shows an A2E increase of more than 3-fold compared with the other three strains. *p < 0.05 in DKO rd8 compared with age-matched C57BL/6J, C57BL/6N and Crb1rd8.

Atrophy. Contrasting, RPE histology is relatively preserved in Crb1rd8 mice and in patients with Leber congenital amaurosis (LCA), a disease associated with Crb1 mutation, even in the advanced stages. These observations suggest that Crb1-associated and DKO rd8-associated pathologies may not operate through identical mechanisms.

RPE apoptosis and neurodegeneration. Compared with age-matched C57BL/6N mice, a 3- to 4-fold increase of ocular A2E is seen in DKO rd8. Contrastingly, Crb1rd8 and C57BL/6J mice do not show elevated A2E levels (Fig. 3). The generation of A2E is considered to be the consequence of burst oxidative stress that is not related in mechanism to the function of Crb1, a component of the molecular scaffold.

Immunopathology of DKO rd8

The DKO rd8 model expresses a spectrum of immunological features of AMD. Increased deposition of complement factors, altered secretion of chemokines and cytokines, and the accumulation of activated microglia and macrophages in retinal lesions have been linked to human AMD and can correspondingly be demonstrated in the DKO rd8. Expression of CD46, a regulatory protein of complement proteins C3 and C4, suggests the presence of activated complement.

Elevated expression of C3d is seen in
RPE and photoreceptors of DKO rd8 and the recruitment of microglia and macrophages in subretinal and retinal lesions is also observed.\(^{23}\) Consistent with AMD patients, DKO rd8 mice demonstrate increased expression of anti-retinal autoantibodies.\(^{23}\)

Moreover, the DKO rd8 strain shows greater macrophage infiltration and complement factor deposition than Crb1\(^{-/-}\) or C57BL/6N (Fig. 4A and B), suggesting that immune dysfunction or activation of innate immunity, which is documented in human AMD, seen in DKO rd8 cannot reasonably be accounted for by the rd8 mutation alone. These results suggest that the immunopathology seen in DKO rd8 should not be thought of as solely secondary immune response to rd8-associated degeneration, a finding that was maintained in the Ccl2\(^{-/-}\)/Cx3cr1\(^{-/-}\) double knockout (Heping Xu, EVER abstract, 2012). Consistent with AMD patients, DKO rd8 mice demonstrate increased expression of anti-retinal autoantibodies.\(^{21}\)

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lesions in retinal inner and outer segment layer, suggesting that Cdx2 and Cx3cr1 double deficiencies could play a role in the high penetrance of rapid photoreceptor and RPE degeneration seen in DKO rd8. Intervention studies conducted on DKO rd8 show reduction or stabilization of these deeper retinal lesions but may have little or no effect on rd8 lesions in the ELM.8-11 Studies of an independently-generated Cdx2+/−/Cx3cr1−/− strain by Luhmann et al.12 indicated DKO rd8 as a model for AMD, citing the primary role of rd8 in the observed early onset retinal degeneration.9 While the rd8 mutation may contribute critically to photoreceptor degeneration, the RPE pathology, immune dysfunction, and A2E accumulation as manifested in DKO rd8 still require further explanation. Thus, interaction between Cdx2, Cx3cr1 and rd8 originated from C57BL/6N is possible and further investigation is needed to elucidate the exact mechanisms and how they fit in to a model for AMD.

**DKO rd8 as a Model for AMD**

When discussing the implications of the rd8 mutation in the DKO rd8 mouse model, consideration must be given to the influence of the genetic background of any engineered mouse model for disease. The generation of knockout mice using 129 and C57BL/6N strains exposed the model to the possibility of “passenger” genes, residual regions of 129-derived genetic material that were transported along with the “knocked out” gene onto C57BL/6N background, having created potential sites of genetic variability.13 Genetic confounders arising from traditional knockout technology using 129 and C57BL/6 may have substantial impact on phenotype, as the two mouse strains have significant genetic and physiological differences and 129 is known to have a multitude of its own mutations.14

Currently, there is no clear model for recapitulating the full clinical features of AMD. It must be taken into consideration that mice lack macula and thus, no murine model can ever fully recapitulate human AMD. Oxidative damage models dependent on photooxidation or high fat diet alone or in combination do not show all characteristic RPE changes, increased levels of A2E or complement deposition.25,26 Induced choroidal neovascularization using matrigel, laser photocoagulation or elevated VEGF expression fail to model mechanisms behind the presentation of disease and thus do not result in increases of A2E or complement deposition. Immunization with carboxyethylpyrrole- (CEP)-adducted protein in mice shows RPE alteration and complement deposition, yet retinal lesions independent of macrophage accumulation are observed and choroidal neovascularization does not develop in this model.27-30 Nonetheless, a growing body of evidence implicating AMD as an immunological disease necessitates that any mouse model featuring “immunopathological” mechanisms.

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