Criteria for Development of Animal Models of Diseases of the Eye

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THE LACK OF AVAILABLE HUMAN OCULAR TISSUES of certain diseases has prompted the use of animals with similar if not identical diseases to man. In some cases, animals with naturally occurring diseases are not readily available for investigation, necessitating the use of animals with induced disease. In other cases, suitable spontaneous models exist and are utilized in studies of the comparable human disease. Animal models of diseases of the cornea, sclera, iridocorneal angle, lens, and retina, to name but a few, have been characterized and have been used to a variable extent on comparative studies of disease mechanisms. Some of these animal models have been briefly described.[1] Because of space limitations, I will consider one human disease and review some of the animal models currently in use.

Human Disease: Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is one of several generalized retinal degenerations grouped under the term primary pigmentary retinal degenerations or rod–cone dystrophies.[2] Ophthalmoscopic examination of affected patients shows pigmentary retinal changes, wax-like disks, and narrow retinal vessels. The pigmentary changes are the hallmark of the disease and appear as spidery accumulations of pigment localized primarily in the midperiphery in the fundus and often seen around the veins. Intraretinal pigmentation, however, is not a prerequisite for diagnosis, and some individuals may show no pigment abnormalities (retinitis pigmentosa *sine pigmento*).[2]

Patients with RP have defective night vision and a visual field defect that is initially recognized in the midperiphery as a complete or incomplete annular scotoma. With progression of the disease the field defect spreads centrally and peripherally, resulting in a marked constriction of the central area of vision (tunnel vision) and possibly blindness. RP is inherited by one of several genetic modes; the severity and rapidity of progression of the disease appear to be dependent on the genetic type.[2]

Initial assessment of retinal function in RP patients by means of elec-

troretinography showed that the electroretinogram (ERG) was extinguished or nonrecordable.[3] This absence of recordable ERG response has been considered by Pearlman[4] as diagnostic a criterion of RP as retinal pigmentary changes, waxy pallor of the optic disk, and arteriolar attenuation.

Most of the ERG studies in RP patients have evaluated retinal function in the late stages of the disease. It is not surprising, therefore, to find nonrecordable or very small amplitude responses in individuals whose retinas show the very advanced atrophic changes characteristic of the disease. Recent ERG studies on young patients have demonstrated that different electrophysiologic defects exist in each genetic type of RP. These studies have been reported by Berson and associates in a series of detailed publications that have been summarized in a symposium published in the Transactions of the American Academy of Ophthalmology and Otolaryngology.[5] Since each genetic type of RP appears to have a distinct functional defect and a characteristic disease course, it is probable that the term RP groups several different diseases that appear clinically similar.

Histopathologic studies of retinitis pigmentosa have been limited because of lack of adequately fixed and processed tissues at an early enough stage of the disease. In the eye from a 64-year-old patient, Verhoeff[6] reported the presence of large broad cone photoreceptors in the zone between the macula and optic disk. In other areas the photoreceptors had disappeared, leaving the external limiting membrane in contact with the pigment epithelium. The appearance of such advanced photoreceptor disease in the posterior pole appears to be quite characteristic of the late stages of RP.[7]

In order to properly understand the disease, it is necessary to obtain postmortem eyes from RP patients whose disease has been well characterized. By means of the Eye Donor Program operated by the Retinitis Pigmentosa Foundation, it is now possible to obtain fresh cadaver eyes from patients for morphologic, biochemical, and tissue culture and other studies. The efforts have proved quite successful, and an increasing number of donor eyes have been subjected to very rigorous scientific investigation. It is anticipated that a greater understanding of this progressively blinding human disease will result from these studies. Until more is known about the human disease, however, studies in animals with retinal disease will continue.

Animal Models of Retinitis Pigmentosa

Hereditary retinal degenerations have been recognized in a number of different animal species (Table 1). Each disease has its own characteristic

clinical, functional, histopathologic, and biochemical features. Although none show on ophthalmoscopy the intraretinal pigmentation character-istic of RP, all show retinal thinning, vascular attenuation, and, in some cases, waxy pallor of the optic disk. The criteria for their inclusion as ani-mal models of RP is based primarily on their being progressively blinding retinal degenerations. Since our understanding of the pathogenesis of RP is fragmentary, studies of the retinal diseases in each animal model pro-vide information on the different mechanisms of disease that affect the vi-sual cells. Selected inherited retinal diseases in rats, mice, and dogs will be briefly reviewed below.

The Rat

Inherited retinal degeneration in the rat was initially reported by Bourne and associates.[8] The disease is inherited as simple recessive, and there is a progressive degeneration of the visual cell layer until blindness results. Most studies since the report by Bourne and associates have used the tan-hooded pink-eyed Royal College of Surgeons (RCS) strain.[9]

In normal rats the retina is not fully developed at birth. In the first 2 weeks of life the visual cells begin to differentiate; rod photoreceptor in-ner and outer segments (OS) elongate, and synaptic contacts develop in the plexiform layers.

The rod OS is composed of orderly stacks of membranous disks that are formed at the base of the OS and are displaced sclerad with time by the orderly and continued addition of new disks at the base. Groups of disks at the distal tip of the outer segment are detached and are then engulfed by the pigment epithelium (PE); these groups of disks are readily visualized in the PE (phagosomes) and are subsequently degraded by the lysosomal system. This process of disk addition and loss, ie, outer segment renewal, is balanced in the developed retina. This prevents large fluctuation in the length of the outer segment in normal animals kept under standard labo-ratory conditions under cyclic lighting. During normal postnatal retinal differentiation, however, the process of disk addition and loss is not bal-anced; this temporary imbalance in favor of disk addition results in the gradual elongation of the photoreceptor outer segment until it reaches adult dimensions.[10]

In the RCS strain of retinal dystrophic rats there is a defect in the outer segment renewal process. Extracellular OS material begins to accumulate adjacent to the PE at 12 days of age, the time when rod outer segments are beginning to elongate. The amount of extracellular OS material, ie, debris, increases with aging until it reaches a maximum at 2 months of age.

Table 1—Selected Animal Models for Retinitis Pigmentosa

Species	Disease	Strain (breed)	Inheritance	Defect	References[*]
Rat	Retinal dystrophy	RCS†	Simple recessive	Defective pigment epithelial phagocytosis of shed photoreceptor outer segment (OS).Visual cell degeneration is secondary to OS debris accumulation.	9, 11, 12, 14
	Retinal degeneration	Wag/Rij	Dominant?	Early onset, slowly progressive degeneration of rods and cones. Lesion evident in cytoplasm of inner segment and perinuclear region. OS initially spared.	32, 33
Mouse	Retinal degeneration (rd)	‡	Simple recessive	Arrested photoreceptor differentiation secondary to abnormal cyclic GMP metabolism in visual cells. Pigment epithelial function is normal.	17, 18, 20
	Retinal degeneration in cerebellar mutant mice	Nervous (nr)	Simple recessive	Slowly progressive photoreceptor degeneration. Partial or complete phagocytic defect by pigment epithelium suspected.	34
	Retinal degeneration in cerebellar mutant mice	Purkinje cell degeneration (pcd)	Simple recessive	Slowly progressive photoreceptor degeneration. Pigment epithelial phagocytic function is normal.	34
Dog	Central progressive retinal atrophy (CPRA)	Labrador retriever, others	Dominant with variable penetrance?	Primary pigment epithelial disease with secondary photoreceptor and retinal atrophy. PE cells become enlarged, accumulate autofluorescent lipopigments, and migrate intraretinally.	24, 35

Progressive retinal atrophy (PRA)				
a) Progressive rod–cone degeneration	Miniature poodle	Simple recessive	Postnatal development of photoreceptors appears normal. Visual cells show vesicular membranous fragments of outer segment, which progress, and photoreceptor degeneration follows. Cone disease less severe than rod disease.	25, 28
b) Rod dysplasia	Norwegian elkhound	Simple recessive	Rod photoreceptors fail to differentiate normally and then degenerate. Cone degeneration is a later event in the disease process.	27
c) Rod–Cone dysplasia I	Irish setters	Simple recessive	Arrested photoreceptor differentiation secondary to abnormal cGMP metabolism in visual cells. Photoreceptor degeneration is rapid.	29, 31, 36
d) Rod–Cone dysplasia II	Collie	Simple recessive	Arrested photoreceptor differentiation. Disease is more extensive and rapidly progressive than rod–cone dysplasia I (Irish setter). The 2 diseases are genetically distinct.	30, 37

* References are not inclusive but reflect key or review articles for each of the diseases.
† Congenic strains available.
‡ Retinal degeneration (rd) mutation is present in several inbred and noninbred stocks of mice.[9]

Autoradiographic studies of outer segment renewal in the RCS rat have demonstrated a failure by the pigment epithelium to phagocytize shed rod outer segment disks.[12,13] This results in the excessive accumulation of OS debris and secondary photoreceptor degeneration.[9] Recent studies with experimental chimeras [14] and *in vitro* with pigment epithelial cells from dystrophic animals [15] have confirmed that the phagocytic defect resides in the pigment epithelium.

Retinal dystrophy in the rat is an early-onset retinal degeneration. Structural abnormalities are evident early in the postnatal period and rapidly progress to complete visual cell degeneration and loss. The similarity of the disease in the rat to RP in man is yet to be determined. The disease, however, illustrates a unique mechanism of photoreceptor degeneration, which results from a disturbance in the control of rod outer segment renewal.

The Mouse

The retinas of mice affected with the recessively inherited retinal degeneration (rd) mutation are normal at birth. Photoreceptor inner segments begin to extend through the external limiting membrane, and by 9 days of age the retina is indistinguishable from that of normal controls. Soon after the appearance of the developing outer segments, the visual cell layer begins to degenerate. The degenerative process is rapid, and by 20–30 days most of the photoreceptors and their nuclei have disappeared.[9,16,17]

The disease in the rd mouse is one of arrested development; photoreceptors begin to degenerate during postnatal differentiation. At no time do the visual cells develop to maturity before degenerating. Unlike the RCS rat, there is no abnormality in the shedding and disposal aspects of the outer segment renewal process. Similarly, studies with experimental chimeras have demonstrated that the disease is primary to the photoreceptors rather than the pigment epithelium. In the chimeric animals, photoreceptor degeneration occurs in association with both mutant as well as normal pigment epithelium.[18]

Biochemical studies in normal retinas have demonstrated the presence of cyclic guanosine monophosphate (cGMP) in visual cells. The levels are controlled by the enzymes involved in the synthesis (guanyl cyclase) and hydrolysis (cGMP-phosphodiesterase [cGMP-PDE]) of this nucleotide and can be modulated by light.[19]

In the normal mouse retina, a high-K_m cGMP-PDE has been found in association with the photoreceptor layer. In rd mice, on the other hand, deficient activity of this enzyme causes a marked elevation in the levels of

cGMP within the retina. This biochemical defect occurs early during postnatal retinal differentiation and precedes any morphologic evidence of degeneration.[20] With aging, the levels of cGMP rise and reach a maximum when the photoreceptor degeneration is most severe. Corresponding with the loss of visual cells from the retina, the levels of cGMP fall. The photoreceptor damaging effects of cGMP have been confirmed *in vitro* in both Xenopus eye rudiments [21] and, more recently, with human retinal tissues.[22]

The Dog

Hereditary degenerations of the dog retina, termed progressive retinal atrophies, are subdivided into the generalized and central forms.[23] The central disease (central progressive retinal atrophy [CPRA]) is a pigment epithelial dystrophy,[24] while generalized progressive retinal atrophy (PRA) is a disease of the visual cell layer.[25]

PRA is recognized in a number of different dog breeds,[23] although it has been well characterized only in a few. In these breeds PRA is inherited as simple autosomal recessive, and the clinical features of the disease are similar. These include: 1) initial night blindness followed by the progressive loss of day vision until complete blindness develops; 2) ophthalmoscopically visible retinal thinning, narrowing of the retinal vessels, and a pale atrophic optic disk; 3) small amplitude or "extinguished" ERG in animals with readily visible ophthalmoscopic lesions. The clinical appearance of the disease is similar to retinitis pigmentosa.[26]

In each affected canine breed studied, the disease onset and rapidity of progression varies, and this variation is breed specific. This difference suggests that the diseases are pathogenetically different although clinically similar. This has been confirmed by electroretinography. In animals bred to develop the disease, before the onset of ophthalmoscopic abnormalities breed-specific functional deficits have been demonstrated. These ERG abnormalities represent selective photoreceptor lesions to either one or both classes of visual cells and occur at specific times during the life of the animals. This has permitted the further classification of PRA into developmental or degenerative disease.[25,27] Utilizing morphologic and functional criteria we find that in the developmental diseases there is arrested development and subsequent degeneration of one or both classes of photoreceptors. In the degenerative form, on the other hand, photoreceptor degeneration follows apparently normal functional development. The morphologic identity of each disease in each affected breed has also been confirmed.[25,27-29]

In order to establish the genetic identity of each disease, selected mat-

ings between affected dogs of different breeds have been performed (miniature poodle × Norwegian elkhound; Irish setter × Norwegian elkhound; setter/elkhound × collie). In all cases the progeny showed normal retinal differentiation, structure, and function.[25,30] This further demonstrates that, at least in these 4 breeds, the genes controlling each retinal disease are nonallelic.

During our studies with the type of PRA found in the Irish setter (rod-cone dysplasia I) we were impressed with the great morphologic similarity between the canine disease and the inherited retinal degeneration (rd) of mice. Although in mice the retinal degeneration progresses very rapidly once established, the pathogenetic sequence and the ultrastructural lesions were very similar to those in the dog. In both, early photoreceptor development was normal until the stage of formation of the rod outer segment. At this time further photoreceptor differentiation was arrested, and the visual cells degenerated.

Biochemical studies in affected Irish setters have shown that there is deficient activity of a photoreceptor-specific cGMP-PDE. In both Irish setters and rd mice, the defect is the same and results in the elevation of retinal cGMP levels followed by visual cell degeneration and blindness.[31]

Conclusion

The different animal models of retinitis pigmentosa share with their human counterpart a progressive and eventually blinding disease of the photoreceptor layer. The diseases are different, not only in each species but also in different strains within a species. This variety is desirable, since it reveals the number of different genetic loci that control the differentiation and maintenance of normal retinal structure and function. The mechanisms of visual cell disease are, therefore, also varied. Insight into these variations may permit a modification or an arrest of the disease process in order to preserve vision. It is significant that the same biochemical abnormality exists in 2 very different animal species with early-onset retinal degeneration. This raises the possibility that other species, including man, may share the same or similar defects.

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