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ALTERED RHYTHM OF PHOTORECEPTOR OUTER SEGMENT PHAGOCYTOSIS IN $\beta 5$ INTEGRIN KNOCKOUT MICE

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1. INTRODUCTION

In the retina photoreceptor and retinal pigment epithelial cells (RPE cells) are in close contact. The outer segment portions of photoreceptors consist of stacked membranous disks containing the phototransduction machinery. These disks are permanently produced thereby lengthening outer segments. To maintain constant outer segment length photoreceptors eliminate their most aged tips by daily shedding (Young, 1967).

RPE cells form a polarized monolayer. They extend apical microvilli that ensheath photoreceptor outer segments. Outer segment shedding by photoreceptors precedes a burst of phagocytosis by the RPE that efficiently clears photoreceptor outer segment fragments (POS) from the subretinal space and recycles their components (Young and Bok, 1969). POS shedding and subsequent phagocytosis by RPE cells are crucial for photoreceptor cell function and survival. A single gene defect in the *mer* gene encoding the receptor tyrosine kinase Mer abolishes efficient POS phagocytosis by RPE cells in the Royal College of Surgeons (RCS) rat strain (D'Cruz et al., 2000; Nandrot et al., 2000). Failure of RCS RPE to ingest POS causes debris accumulation and rapid photoreceptor degeneration illustrating the importance of RPE phagocytosis (Mullen and LaVail, 1976).

RPE cells do not usually divide in the adult retina. Each RPE cell faces around 30 outer segments depending on the area of the retina. As photoreceptor shed ~7% of their outer segment mass each day, every RPE cell digests 25,000 to 30,000 disks each and every day of life (Besharse and DeFoe, 1998). Therefore, RPE cells are the most active phagocytes in the body. Synchronized POS clearance is tightly regulated and any delay in completing the shedding or digestion process can cause accumulation of undigested material. Indeed, autofluorescent inclusion bodies commonly accumulate in human RPE cells of age. These lipofuscin storage bodies contain a complex mix of proteins and lipids and likely results from incomplete turnover of POS material (Feeney, 1978). *In vitro* studies have recently shown that lipofuscin components may directly impair RPE function and viability (Holz et al., 1999; Finnemann et al., 2002). These data suggest that defective digestion of POS by RPE cells may contribute to development or progression of age-related retinal diseases such as age-related macular degeneration.

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Outer segment renewal in higher vertebrates is synchronized by circadian rhythms influenced by the daily dark-light cycle (Goldman et al., 1980). Animal studies in rod- or cone-dominant species revealed that rods mainly shed their POS ~2 hours after onset of light and cones shed ~2 hours after dusk (LaVail, 1976; Young, 1977). The increase in the number of phagosomes present in RPE cells at these two time points suggests a peak in phagocytic activity every 12 or 24 hours for RPE cells depending on whether they serve rods, cones or both. No untimely phagocytosis has been observed so far, suggesting that RPE cells may downregulate their phagocytic activity if not “on duty”. Currently, mechanisms that regulate RPE phagocytosis are only poorly understood.

2. THE PHAGOCYTTIC MACHINERY OF THE RPE

Three plasma membrane receptors have been shown to fulfill distinct roles in RPE phagocytosis. These are the Mer tyrosine kinase receptor MerTK (D’Cruz et al., 2000; Nandrot et al., 2000), the scavenger receptor CD36 (Ryeom et al., 1996), and the adhesion receptor $\alpha v\beta 5$ integrin (Finnemann et al., 1997; Miceli et al., 1997; Lin and Clegg, 1998). MerTK is involved in the internalization step of phagocytosis (Edwards and Szamier, 1977). MerTK deficient macrophages have a reduced capacity to phagocytose apoptotic cells confirming that OS phagocytosis by the RPE is a mechanism similar to the macrophage clearance mechanism for apoptotic cells (Finnemann and Rodriguez-Boulan, 1999; Scott et al., 2001). *In vitro* experiments have shown that CD36 ligation regulates the rate of POS internalization suggesting a role for CD36 in phagocytic signaling (Finnemann and Silverstein, 2001).

$\alpha v\beta 5$ expression at the apical surface of rodent RPE *in vivo* coincides with postnatal establishment of mature interactions between photoreceptors and RPE including the onset of POS renewal (Ratto et al., 1991; Finnemann et al., 1997). Stable binding of POS to the cell surface of RPE cells in culture is largely dependent on $\alpha v\beta 5$ integrin receptors (Finnemann et al., 1997). In addition to POS recognition or tethering RPE cells also use $\alpha v\beta 5$ integrin receptors to activate signaling pathways through focal adhesion kinase that are necessary to activate MerTK (Finnemann, 2003; Nandrot et al., 2004; and see chapter by Finnemann and Nandrot in this volume). As $\beta 5$ integrin only dimerizes with αv integrin subunits, $\beta 5$ integrin knockout mice provide the opportunity to study RPE cells that permanently and exclusively lack $\alpha v\beta 5$ receptors.

3. IMPACT OF LACK OF $\alpha v\beta 5$ INTEGRIN ON RPE FUNCTION

$\beta 5$ knockout mice are viable and fertile, and have normal lifespan (Huang et al., 2000). When we first examined their retinal tissues we did not find gross anatomical abnormalities in $\beta 5$ null mice regardless of age (Nandrot et al., 2004). We then set out to compare wild-type and $\beta 5$ knockout RPE phagocytic function both *in vitro* and *in vivo*. Finally, we investigated vision and RPE cell ultrastructure as a function of age in $\beta 5$ knockout and wild-type control mice.

To test POS phagocytosis *in vitro* we isolated and maintained in primary culture RPE cells from $\beta 5$ null and wild-type control animals. Strikingly, $\beta 5$ null RPE cells failed to efficiently take up OS. This decrease was not due to slower uptake kinetics as uptake by $\beta 5$ null cells remained low at all times of phagocytic stimulation (Figure 1 a).

To check POS phagocytosis *in vivo* we counted phagosomes present in RPE cells of 4 week old wild-type and $\beta 5$ null mice at different time points of the 24-hour period. As expected, we detected a peak in the number of phagosomes in wild-type retina ~2 hours after the light onset occurring at 6 AM in our facility (Figure 1 b) (LaVail, 1976). This peak was lost in $\beta 5$ null retina in which we counted equal numbers of phagosomes at all time points tested.

However, phagosome counts in $\beta 5$ null retina were similar or higher than phagosome counts in wild-type retina outside of the phagocytosis peak. Thus, phagocytosis in $\beta 5$ knockout retina is not abolished but loses its temporal regulation. absence of $\alpha v\beta 5$ integrin eliminates the burst of phagocytic activity required for synchronized POS engulfment.

These results show that POS phagocytosis by RPE cells is impaired in 1-month-old $\beta 5$ null mice. However, $\beta 5$ null mice develop impaired vision only at a much older age. Scotopic electroretinograms (ERGs) recorded in 1-year-old animals showed a strongly attenuated response in $\beta 5$ null compared to wild-type mice (Figure 2 a). The reduction of retinal responses to light stimuli was progressive from 4 months of age in $\beta 5$ null animals whereas responses did not vary greatly for wild-type animals (Figure 2 b).

Finally, we detected excessive autofluorescent lipofuscin deposits in RPE of 1-year-old $\beta 5$ null mice by wide-field fluorescence microscopy (Nandrot et al., 2004). These lipofuscin granules appeared as dense opaque bodies on ultrathin sections examined by electron microscopy. We observed few of these inclusion bodies in wild-type RPE (Figure 2 c), whereas they were present in large numbers in $\beta 5$ null RPE (Figure 2 d).

4. PERSPECTIVE

Our results show that lack of $\alpha v\beta 5$ integrin receptors eliminates the rhythm of POS phagocytosis in the retina. With age, mice lacking $\alpha v\beta 5$ integrin progressively lose vision and their RPE accumulates lipofuscin granules. Strikingly, even with an early phagocytosis defect $\beta 5$ null mice only develop late onset pathology. Our findings suggest that constant instead of rhythmic POS phagocytosis by RPE cells may impair POS digestion causing gradual accumulation of autofluorescent compounds as lipofuscin.

Taken together these data emphasize the importance of timely regulation of RPE phagocytosis. $\alpha v\beta 5$ integrin activity synchronizes this daily process that is essential for vision (Nandrot et al., 2004). The age-related changes we observed in retinas of $\beta 5$ knockout mice share some characteristics of retinas of humans with age-related macular degeneration. The $\beta 5$ knockout mouse strain may thus provide a useful animal model to study lipofuscin buildup with age and gene therapies to delay or reverse its accumulation.

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Figure 1.

$\beta 5$ integrin deficient RPE cells display defects in POS phagocytosis. **(a)** After 1 to 3 hours of POS phagocytic challenge *in vitro*, $\beta 5^{-/-}$ cells in primary culture phagocytosed less OS than $\beta 5^{+/+}$ cells. **(b)** Phagosomes were counted on cross sections of $\beta 5^{+/+}$ and $\beta 5^{-/-}$ mice eyecups at different time-points of the 24-hour phagocytic cycle. The phagocytic peak detected in $\beta 5^{+/+}$ animals 2 hours after the light onset is absent in $\beta 5^{-/-}$ mice. Modified from Nandrot *et al.* (2004), with permission from Rockefeller University Press.

Figure 2.

Age-related retinal changes in $\beta 5^{-/-}$ mice. **(a)** At 1 year of age, scotopic ERG responses are greatly reduced in $\beta 5^{-/-}$ mice. **(b)** ERGs were recorded for wild-type and $\beta 5^{-/-}$ mice between the ages of 4 months and 1 year. Responses declined after 4 months in $\beta 5^{-/-}$ animals, both the a- and b-waves were affected. RPE cells of 1-year-old wild-type **(c)** and $\beta 5^{-/-}$ **(d)** animals were examined by electron microscopy on ultrathin sections. Electron dense inclusion bodies were detected in $\beta 5^{-/-}$ RPE in larger numbers than in wild-type RPE. Modified from Nandrot et al. (2004) with permission from Rockefeller University Press.