Lack of v 5 Integrin Receptor or Its Ligand MFG-E8: Distinct Effects on Retinal Function

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Lack of αvβ5 Integrin Receptor or Its Ligand MFG-E8: Distinct Effects on Retinal Function

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Key Words
Retinal pigment epithelium · Photoreceptors · Phagocytosis · Integrin · Ligand · Blindness

Abstract
Background/Aims: Diurnal phagocytosis of spent photoreceptor outer segment fragments by the retinal pigment epithelium (RPE) is critical for vision. We recently identified an important role for αvβ5 integrin receptors and their ligand Milk fat globule-EGF factor 8 (MFG-E8) in RPE phagocytosis. ***Methods:*** We compared RPE phagocytosis and retinal function between mice deficient in αvβ5 integrin receptors and mice deficient in the secreted integrin ligand MFG-E8. ***Results:*** Both $\beta5^{-/-}$ and MFG-E8$^{-/-}$ mice exhibit the same phagocytic defect: RPE cells retain basal uptake activity but completely lack the burst of phagocytic activity as well as the rhythmic activation of Mer tyrosine kinase that follow circadian photoreceptor shedding in wild-type RPE. Strikingly, electroretinogram photoresponses decline with age only in $\beta5^{-/-}$ but not in MFG-E8$^{-/-}$ retina. ***Conclusion:*** These results identify a critical role of αvβ5 integrin receptors and their ligand MFG-E8 in synchronizing retinal phagocytosis. Additionally, we show that lack of αvβ5 receptors and MFG-E8 ligand have distinct consequences for retinal function. These intriguing results suggest that loss of phagocytic rhythm is not solely responsible for the age-related blindness of $\beta5^{-/-}$ mice.

Introduction

Photoreceptors continuously renew their light-sensitive outer segments and shed their distal tips daily [1]. It is the task of the adjacent retinal pigment epithelium (RPE) to efficiently phagocytose shed photoreceptor outer segment fragments (POS) and to recycle or digest their components [2]. Outer segment renewal in higher vertebrates is synchronized by circadian rhythms [3]. In mice and rats, rods shed following light onset [4]. As each mammalian RPE cell is postmitotic and faces ~30 outer segments, RPE cells are the most active phagocytes in the body.

Outer segment renewal is crucial for photoreceptor function and survival. Lack of efficient POS phagocytosis by RPE cells in the Royal College of Surgeons rat strain is sufficient to cause rapid photoreceptor degeneration [5]. Delay in POS digestion can directly cause accumulation of undigested POS material as lipofuscin in the RPE [6], which is detrimental to RPE and retina and may contribute to the development or progression of age-related macular degeneration [7–9].

The molecular mechanism RPE cells use to phagocytose POS is only partially understood. Engulfment of POS strictly requires Mer tyrosine kinase (MerTK), whose mutation causes retinal dystrophy in the Royal College of Surgeons rat [10–12]. We have recently identified an important role for the receptor-ligand pair αvβ5 integrin-

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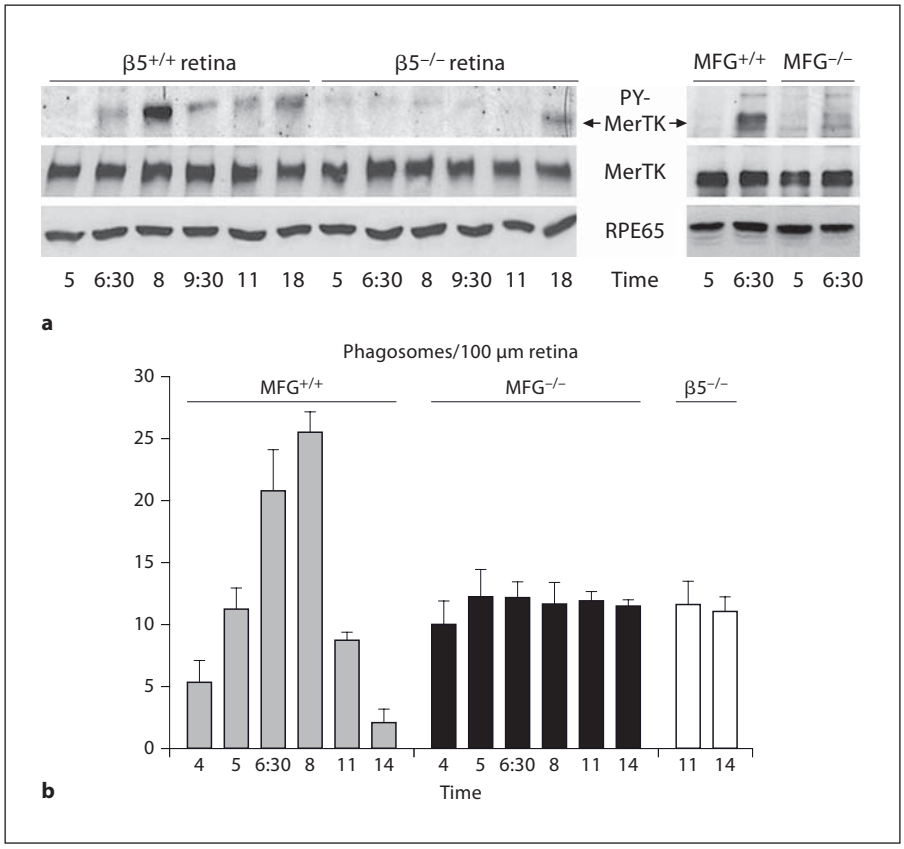

Fig. 1. αvβ5- and MFG-E8-deficient RPE cells equally lack the daily rhythm of POS phagocytosis. **a** Both MFG-E8$^{-/-}$ and β5$^{-/-}$ RPE lack peak MerTK phosphorylation (PY-MerTK) 30 min after light onset, although MerTK protein levels are normal (MerTK). **b** Both MFG-E8$^{-/-}$ and β5$^{-/-}$ RPE lack peak phagosome residence found in wild-type RPE ($^{+/+}$) 2 h after light onset. Modified from Nandrot et al. [13], with permission from Rockefeller University Press, and from Nandrot et al. [14], with permission from the National Academy of Sciences.

MFG-E8 (Milk fat globule-EGF factor 8) in RPE phagocytosis in vivo upstream of MerTK [13, 14]. Here, we directly compare how loss of either αvβ5 receptor or MFG-E8 ligand in mice affects retinal function.

Materials and Methods

Animals

MFG-E8$^{-/-}$ and control MFG-E8$^{+/+}$ [14], as well as β5$^{-/-}$ [13] and wild-type mice all with the same genetic background (129T2/SvEmsJ) were housed under cyclic 12-hour light:12-hour dark conditions (light onset at 6.00 h) and bred. All procedures involving animals were approved by the Weill Medical College Institutional Animal Care and Use Committee.

Electroretinography

Electroretinograms of background- and age-matched mice were recorded monthly from 4 to 12 months of age as described previously [13]. Full-field scotopic electroretinograms were recorded on dark-adapted, anesthetized mice using a custom-made gold wire corneal contact lens electrode as well as subdermal reference and ground electrodes. A photostimulator in a Ganzfeld reflective dome was used to deliver 10-μs white flashes (UTAS-2000, LKC Technologies). Neutral density filters from –2.4 to 0 log neutral density filter in 0.4 log unit steps were used to decrease full-intensity flash stimuli of 1.5 cd s/m^2. At least 3 separate responses were recorded and amplitudes averaged for each intensity.

Phagosome Quantification, Tissue Lysis and Comparative Immunoblotting

The phagosome counts and immunoblots shown in figure 1 are reproduced in modified form from Nandrot et al. [13, 14]. Experimental details are described in the original publications.

Results

Mice Lacking αvβ5 Integrin Receptors or Their Ligand MFG-E8 Share the Same Loss of RPE Phagocytic Rhythm

We previously studied RPE phagocytosis in 2 mutant mouse strains that specifically lack either αvβ5 integrin (an adhesive receptor known to localize to the apical RPE surface [15]) or the secreted glycoprotein MFG-E8 (known to bind to integrins via its RGD motif as well as to phosphatidylserine phagocytic 'eat me' signals of apoptotic

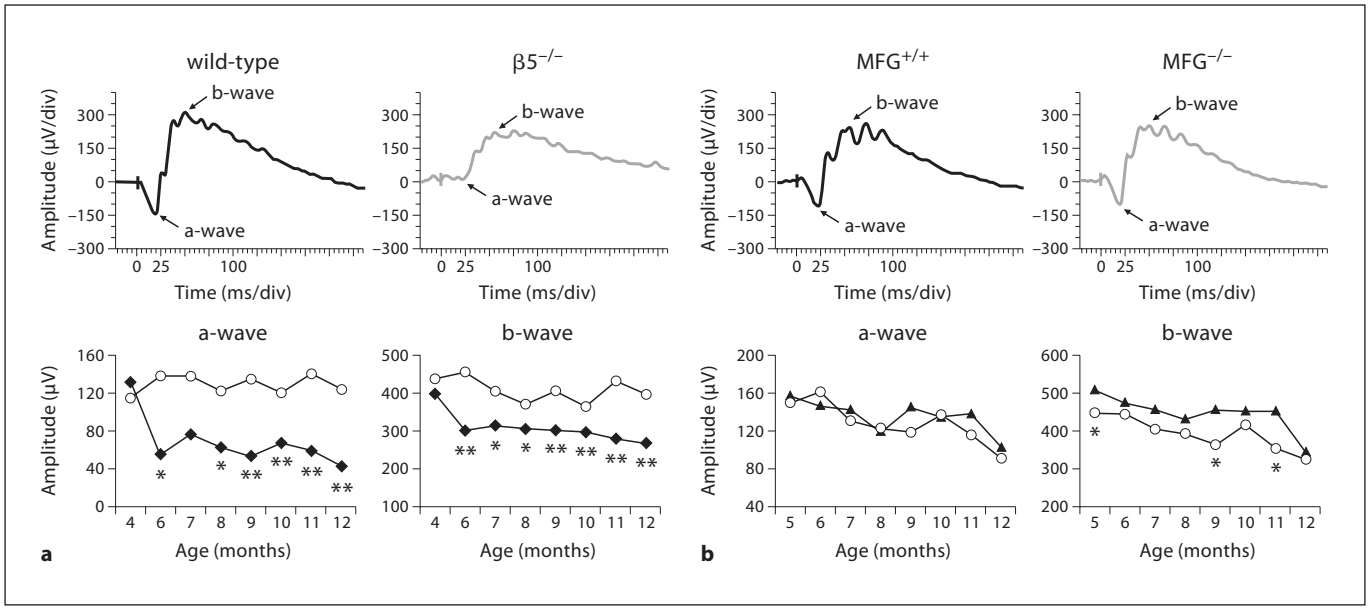

Fig. 2. β5$^{-/-}$ but not MFG-E8$^{-/-}$ mice lose vision with age. Electroretinograms were recorded for wild-type and β5$^{-/-}$ (**a**) as well as for MFG-E8$^{-/-}$ and strain-matched control (**b**) mice between the ages of 4 months and 1 year. **a** At 1 year of age, scotopic electroretinogram responses of β5$^{-/-}$ mice are significantly weaker than responses by wild-type mice. Both a- and b-wave amplitudes declined from 4 months of age in β5$^{-/-}$ mice (black diamonds). Modified from Nandrot et al. [13], with permission from Rockefeller University Press. **b** Up to 1 year of age, scotopic electroretinogram responses of MFG-E8$^{-/-}$ mice (dark grey triangles) do not significantly differ from responses of control mice (white circles). Student's *t* test, n = 4–7, * $p < 0.05$, ** $p < 0.01$.

cells and POS [16, 17]). We found that both receptor- and ligand-deficient mice failed to increase retinal MerTK phosphorylation following light onset (fig. 1a). Furthermore, they both lacked the synchronized burst of diurnal RPE phagocytosis and instead displayed equal phagocytic activity at all times of day [13, 14] (fig. 1b).

Unlike Mice Lacking αvβ5 Integrin Receptors, Mice Lacking αvβ5 Ligand MFG-E8 Do Not Lose Visual Function with Age

We previously demonstrated that a dramatic decline in photoreceptor function (fig. 2a) correlates with accumulation of excess autofluorescent RPE lipofuscin in aging αvβ5-receptor-deficient mice [13]. Therefore, we hypothesized that mice lacking the αvβ5 ligand MFG-E8 may also show age-related retinal dysfunction. However, we found that MFG-E8-ligand-deficient mice retain similar vision as background-matched control mice until 1 year of age (fig. 2b). These data clearly demonstrate that loss of phagocytic rhythm alone is not sufficient to cause age-related blindness.

Discussion

The identical arrhythmic POS phagocytosis and lack of MerTK activation following light onset in MFG-E8- and αvβ5-deficient RPE is consistent with a function of MFG-E8 as the sole ligand for αvβ5 integrin in retinal phagocytosis. We speculate that varying availability of MFG-E8 in the interphotoreceptor matrix may rhythmically activate apical αvβ5 receptors of the RPE to synchronize diurnal POS clearance.

Age-related changes in RPE and neural retina develop secondarily and by yet unknown mechanisms in mice lacking αvβ5 receptors. Because MFG-E8-deficient mice do not recapitulate these changes, we speculate that αvβ5-deficient retina may harbor defects in addition to the loss of phagocytic rhythm. Notably, we previously showed that lack of αvβ5 but not lack of MFG-E8 greatly decreases the strength of retinal adhesion [14, 18]. It is thus conceivable that it is the combination of aberrant time course of POS phagocytosis and permanently weakened outer segment adherence to the underlying RPE that causes the age-related decline in photoreceptor function

in mice lacking αvβ5. Identification of the αvβ5 ligand responsible for promoting retinal adhesion and generation of double-mutant mice lacking both phagocytic and adhesive ligands for αvβ5 may allow direct testing of this hypothesis in the future.

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