Early Activation of Inflammation- and Immune Response–Related Genes after Experimental Detachment of the Porcine Retina

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Purpose. To determine early alterations in retinal gene expression in a porcine model of rhegmatogenous retinal detachment.

Methods. Local detachment was created in eyes of adult pigs by subretinal application of sodium hyaluronate. The gene expression in control tissues and retinas detached for 24 hours was analyzed with a pig genome microarray. Genes with at least three-fold expression changes were detected in the detached retina and in the attached retinal tissue surrounding the local detachment in situ. Structural alterations of the retina were examined by light and electron microscopy.

Results. Identified were 85 genes that were upregulated and 7 that were downregulated in the detached retina. Twenty-eight genes were identified as upregulated in the nondetached retina of the surgical eyes. The genes upregulated in detached retinas were related to inflammation and immune responses ($n = 52$), antioxidants and metal homeostasis ($n = 7$), intracellular proteolysis ($n = 6$), and blood coagulation/fibrinolysis ($n = 4$). The upregulation of at least 15 interferon-stimulated genes indicates elevated interferon levels after detachment. Gene expression of blue-sensitive opsin was not detectable in the detached retinal tissue, suggesting an early reduction in phototransduction, especially in blue cones. Electron microscopy revealed an accumulation of microglial cells in the inner retinal tissue and of polymorphonuclear leukocytes in the vessels of detached and peridetached retinas.

Conclusions. Differentially expressed genes in the retina early after experimental detachment are mainly related to inflammation and immune responses, intracellular proteolysis, and protection against oxidative stress. A local immune and inflammatory response may represent a major causative factor for reactive changes in the retina after detachment. The inflammatory response is not restricted to the detached retina but is also observed in the nondetached retina; this response may underlie functional changes in these regions described in human subjects. (*Invest Ophthalmol Vis Sci.* 2008;49:1262–1273) DOI:10.1167/iovs.07-0879

Detachment of the neural retina from the pigment epithelium is a major cause of visual impairment after trauma and during ocular inflammation and may be the result of the presence of retinal holes or tears. At present, the only effective treatment of retinal detachment is surgery. However, an early and ophthalmoscopically successful reattachment often fails to restore the normal visual capabilities,[1,2] and patients often describe permanent defects in color vision and a decline in visual acuity.[3,4] Clearly, there is a need for the detection of early functional alterations in the retina after detachment that may stimulate the development of novel therapeutic intervention to be used during reattachment surgery for the support of functional recovery.

Experimental retinal detachment causes complex alterations and cellular remodeling throughout the retina which may underlie the poor recovery of vision after reattachment.[5] The initial deconstruction of the photoreceptor outer segments and the apoptotic death of some photoreceptor cells is associated with the development of reactive changes in the inner retinal neurons[6] and accompanied by fast activation of pigment epithelial and macro- and microglial cells.[7–9] In addition, retinal detachment causes changes in the retinal blood supply, resulting in prolonged retinal circulation times.[10,11] The response of glial cells is initiated within minutes of experimental detachment and develops during the first hours and days after creation of detachment.[8,12,13] However, the pathogenic factors that induce the early responses of retinal cells to detachment are poorly investigated. It has been shown that delivery of high oxygen during detachment reduces the gliotic responses and the photoreceptor cell degeneration, suggesting that hypoxia of the retinal tissue is a major pathogenic factor.[14,15] In a recent study of gene expression in the detached retina of the rat, the early activation of stress response genes and of distinct signaling pathways suggests that oxidative stress is a major factor that triggers cellular responses to detachment.[16]

Previous animal studies indicate that rhegmatogenous retinal detachment is associated with a local inflammatory response. In addition to activation of microglial cells and infiltration of macrophages into the subretinal space, an increase in the retinal expression of cyclooxygenase immunoreactivity has been described.[17–19] An increase in gene and protein expression of various proinflammatory factors such as tumor necrosis factor, interleukin-1β, and monocyte chemotactic protein (MCP)-1, in the neural retina of the rat was observed within 6

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hours after detachment.[20] To characterize the inflammatory response of the neural retina early after detachment more precisely, we investigated the gene expression in porcine retinas detached for 24 hours. We found that, in addition to genes implicated in oxidative stress response and intracellular proteolysis, genes for factors involved in inflammation, immune responses, and blood coagulation/fibrinolysis are increased in their expression. This finding suggests that a local immune and inflammatory response represents a major causative factor for reactive changes in the retina after detachment. An electron microscopic investigation revealed that reactive microglial cells in the retinal parenchyma, as well as leukocytes accumulated in retinal vessels, may contribute to the enhanced retinal expression of inflammatory and immune response genes.

Retinal detachment causes visual impairment in the detached region, and frequently, also in nondetached regions that surround the local detachment.[21] Visual impairment and color confusion may also include the macula in cases with purely peripheral detachment.[1,4,22] Optical coherence tomography performed before reattachment surgery often demonstrated edema in the macular tissue,[23,24] even in cases when the macula remained attached.[25] The retinal blood circulation times of the detached areas are longer than those of the nondetached regions, and both are longer than those of normal subjects.[10] These observations in human subjects correspond with findings in animal models of local retinal detachment suggesting that, in addition to reactive changes in the detached retina, there are gliotic alterations and photoreceptor degeneration also in the surrounding nondetached tissue, albeit at lower levels than in the detached tissue.[12,18,19,26,27] To determine whether early alterations in gene expression also occur in nondetached retinal areas of the surgical eyes, we compared the gene expression responses in the detached and attached retinal areas of the porcine eye.

Materials and Methods

Surgical Procedure

All experiments were performed in accordance with applicable German laws and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Four young adult domestic white pigs (17–22 kg; both sexes) were used to obtain the gene expression data with a microarray (Affymetrix, Santa Clara, CA) and a real-time PCR analysis in whole neural retinas and pigment epithelial cells and for the production of retinal sections. Two further animals (19–20 kg; both sexes) were used for a real-time PCR analysis of Müller cell–enriched fractions. Twenty-four hours before and after surgery, the food intake of the animals was restricted, with free access to water. Intramuscular azaperone (15 mg/kg; Cilag-Janssen, Neuss, Germany), atropine (0.2 mg/kg; Braun, Melsungen, Germany), and ketamine (3 mg/kg; Ratiopharm, Ulm, Germany) were administered for premedication. By stepwise application of ketamine (5 mg/kg) and propofol (5 mg/kg; Ratiopharm, Ulm, Germany), a totally intravenous anesthesia was performed. Deltajonin (Delta Select, Pfullingen, Germany) was continuously infused via a vein of the ear. Pulse rate and pO_2 were monitored during anesthesia, and O_2 (3 L/min) was applied.

Rhegmatogenous detachment of the retina was created under sterile conditions in one eye of the animals; the other eye served as the nonsurgical control. The pupils of the eyes were dilated by topical tropicamide (1%; Ursapharm, Saarbrücken, Germany) and phenylephrine hydrochloride (5%; Ankerpharm, Rudolstadt, Germany), and a lateral canthotomy was created. Hemostasis was achieved with wet-field cautery. After pars plana sclerotomy, a circumscript vitrectomy was performed in the area of the future detachment, and physiologic saline (Delta Select) was infused into the eye to replace the vitreous. Thin glass micropipettes attached to 250-μL glass syringes (Hamilton, Reno, NV) were used to create a retinal detachment by subretinal injection of saline followed by 0.25% sodium hyaluronate in saline (Healon; Pharmacia & Upjohn, Dübendorf, Switzerland). The retina ventral of the optic nerve head was detached, and the dorsal retina remained attached. After surgery, gentamicin (5 mg) and dexamethasone (0.5 mg) were injected subconjunctivally. The lateral canthotomy was closed with 5-0 silk sutures, and atropine (1%) eye drops were instilled into the conjunctival sac. After a survival time of 24 hours, the animals were anesthetized as described, the eyes were excised, and the animals were killed by IV embutramid mebezonium iodide (T61, 0.65 mL/kg body weight; Hoechst, Unterschleissheim, Germany).

Müller Cell Enrichment

The eyes were opened by a circumferential cut posterior to the ora serrata, and the retinas were removed. Pieces of detached and attached (3–4 mm from the border of the circumscribed local detachment) retina and the corresponding control areas were transferred to ice-cold phosphate-buffered saline (PBS; Invitrogen-Gibco, Paisley, UK) and washed three times to remove loosely adherent material. After treatment with papain (40 μL/mL, Roche, Mannheim, Germany) at 37°C for 30 minutes, the enzyme was removed by washing the tissues three times with PBS. Thereafter, the tissues were incubated in PBS (5 mL) containing DNase I (200 U/mL; Sigma-Aldrich, Taufkirchen, Germany) at room temperature for 5 minutes and then triturated through a wide-pore pipette. The tissue fragments were permitted to settle on the bottom of 15-mL tubes for 10 minutes. The supernatant (4 mL) was removed and stored on ice, and PBS (4 mL) was added to the tissue fragments. This procedure was repeated until the retinal cells in the tissue fragments were completely dissociated. To enrich the Müller cells, the supernatants were filtered using a nylon cell strainer (pore size, 40 μm; BD Biosciences, San Jose, CA). The result of each filtration step was checked by phase-contrast microscopy, and the filtration was repeated when the number of Müller cells in the passaged fluid was high. Thereafter, the filters were washed several times with PBS to collect the Müller cells. After a short centrifugation (400*g*, 5 minutes), the Müller cell–enriched fractions were used for total RNA preparation. Apparently, the fractions contained only Müller cells (as recognized on the basis of their characteristic bipolar shape) and cell nuclei, since neuronal cells were damaged during the dissociation procedure, and cells smaller than 40 μm passaged through the filters. No endothelial cells or pericytes/smooth muscle cells were present in the Müller cell–enriched fractions. We determined the percentage of Müller cells in the fractions by counting the Müller cells and cell nuclei and found the following values (means ± SEM): control retinas, 62% ± 7%; detached retinas, 63% ± 4%; nondetached retinas of the surgical eyes, 61% ± 6%.

Total RNA Preparation

Total RNA was prepared from neural retinas, from the pigment epithelium underlying the detached and peridetached retinas in situ, and from Müller cell–enriched cell preparations. The tissues were precipitated by centrifugation at 4°C and washed twice with prechilled PBS. RNA was extracted (TRIzol reagent, 1 mL; Invitrogen-Gibco) and purified (RNeasy Mini Kit; Qiagen, Hilden, Germany). The quality of the RNA was analyzed by agarose gel electrophoresis. The A_{260}/A_{280} ratio of optical density was measured (GeneQuantpro device; Pharmacia) and was between 1.9 and 2.1 for all RNA samples, indicating sufficient quality.

Oligonucleotide Microarray Analysis

The mRNA expression in the total RNA preparations was analyzed by using a gene microarray (GeneChip Porcine Genome Array of the Genechip System; Affymetrix), according to the manufacturer's instructions. This microarray contains probes representing 16,381 full-length genes and expressed sequence tags. Each gene analyzed was represented by 16 to 20 different oligonucleotide probe pairs, with each probe pair consisting of a matched and a mismatched oligonucleotide. The mismatched probes, which served as the control for the

TABLE 1. Primer Pairs Used for PCR Experiments

Gene and Accession	Primer Sequence (5′→3′)	Amplicon (bp)
pGAPDH	GGGCATGAACCATGAGAAGT (s)	230
AF017079	AAGCAGGGATGATGTTCTGG (as)	
pLYZ	AAAAGGAGCAGAATGCCAGA (s)	242
NM-214392	TTCAGGAAAAGCACAGCTCA (as)	
pMCP-1	CCCCGAGAATCTGAAGACAA (s)	210
NM_214214	AAAGCAATGTGCCCAAGTCT (as)	
pSLADRA1	GCCCAACATCCTCATCTGTT (s)	245
AY285933	GAGCTTCAAACTCCCAGTGC (as)	
pBMP-2	CTTCAGGTCTTTCGGGAACA (s)	104
AY669080	GAGTTGGCTGTTGCAGGTTT (as)	
pANXA1	CATCAGGCCATGAAGGGTAT (s)	155
CO944050	TCTCCCTTGGTTTTCATCCAG (as)	
pMT-1A	GCTCTCTGCTTGGTCTCACC (s)	211
NM_001001266	ACAGCAGCTGCACTTGTCC (as)	
pMT-2B	GCAAATGCAAAGACTGCAAA (s)	212
AB000794	GCAAACGGGTCAGGTTGTAT (as)	
pTLR2	CAACATCATCGACTCCATCG (s)	159
NM_213761	ATGGGTTCCAGCAGAATGAG (as)	
pTLR4	GACAGGGAAAGCCAATTCAA (s)	200
AB188301	GGGAAAAATGAACCCAGACC (as)	
pAIF-1	GAAACGAATGCTGGAGAAGC (s)	243
AF299326	ACCACAAATCAGGGCAACTC (as)	
pTF	CCAGGTCATCCAAGGAAAAA (s)	163
NM_213785	AACCCCTCAATACCACGTCA (as)	
pAMBN	GGCATTAAAAGCGCTAAGCA (s)	247
NM_214037	GCAGTGTCACATTTCCTGGAT (as)	

s, sense; as, antisense.

determination of background and of nonspecific hybridization signals, included a single base substitution that inhibited the hybridization with the mRNA of the target gene. The levels of mRNA expression were evaluated using the microarray software (Microarray Suite ver. 5.0; Affymetrix), which calculated three detection levels (present, marginal, absent) and the size of the messages, by considering both the intensities of the signals which were emitted from the probe sets and the number of probe pairs in which the perfect match was specific. The RNA abundance was determined based on the average of the differences between perfect-matched and mismatched intensities for each probe family. Gene induction or repression was considered significant when the change in average difference intensity was above threefold. A comparison analysis was performed in which the relative change in abundance was evaluated for each transcript between a baseline (mean levels in control retinas) and an experimental sample (each retina isolated from surgical eyes). The integrity of the used cRNA samples was tested by checking the 5′-to-3′ ratios of the housekeeping genes.

Real-Time PCR

After DNase treatment, cDNA was synthesized with 1 μg of total RNA (RevertAid H Minus First Strand cDNA Synthesis Kit; Fermentas, St. Leon-Roth, Germany). Semiquantitative real-time RT-PCR was performed (Single-Color Real-Time PCR Detection System; BioRad, Munich, Germany) using the primer pairs described in Table 1. The PCR solution contained 1 μL cDNA, specific primer set (1 μM each), and 10 μL SYBR Green PCR (QuantiTect Kit; Qiagen) in a final volume of 20 μL The PCR parameters were initial denaturation and enzyme activation: one cycle at 95°C for 15 minutes); denaturation, amplification, and quantification: 45 cycles at 95°C for 30 seconds, 58°C for 20 seconds, and 72°C for 45 seconds; and the melting curve: 55°C with the temperature gradually increased (0.5°C) up to 95°C. The amplified samples were analyzed by standard agarose gel electrophoresis. The mRNA expression was normalized to the levels of GAPDH mRNA, and the changes were calculated as described.[28]

Semithin Retinal Sections and Electron Microscopy

Pieces of control, detached, and attached retinas of the surgical eyes were fixed with a buffered mixture of 2% glutaraldehyde and 4% paraformaldehyde overnight. The tissues were rinsed, postfixed in 1% osmium tetroxide for 2 hours, dehydrated in ethanol, and stained overnight in 70% ethanol saturated with uranyl acetate. After further dehydration in absolute ethanol and propylene oxide, the samples were embedded with an Araldite 502 kit (Sigma-Aldrich, Steinheim, Germany) and sectioned on an ultramicrotome (Nova; LKB, Bromma, Sweden). Semithin sections (0.5 μm) were stained with toluidine blue and visualized by using a conventional light microscope. Ultrathin sections were studied with an electron microscope (EM 902; Carl Zeiss Meditec GmbH, Oberkochen, Germany).

RESULTS

Studies have revealed that the response of neuronal and glial cells to local detachment is not restricted to the site of detachment but is also obvious (albeit at lower levels) in the attached retina that surrounds the local detachment in situ.[12,18,19,26] Therefore, we determined the gene expression in the detached and peridetached neural retinas of surgical eyes and compared the values with the average gene expression levels of control retinas isolated from nonsurgical eyes. Of the 16,381 gene probes investigated, approximately 11,000 transcripts were expressed in the retina of the pig. In retinas that were detached for 24 hours, the difference in the mean expression level of 254 transcripts was greater than threefold when compared with control retinas and were therefore considered to be up- or downregulated in response to detachment. In attached retinal areas of the surgical eyes, the expression level of 75 transcripts was altered in response to detachment. The identified genes with altered expression in four independent microarray analyses are summarized in Tables 2, 3, and 4. We identified 85 genes that were upregulated in detached retinas (Tables 2, 3). Twenty-eight of these genes were also expressed at higher level in the nondetached retinas of the surgical eyes; however, the increase in the expression level was in most cases lower than in detached retinas. The data suggest that alterations in the gene expression after local detachment are not restricted to detached retinas but are also observed in peridetached retinas surrounding local detachments in situ.

In contrast to the relatively large number of upregulated genes, only a few genes were identified to be downregulated after detachment (Table 4). Downregulation of genes was found only in detached but not in peridetached retinas, with the exception of one gene (IGFBP-5) which was absent also in the peridetached retina. The expression of the zinc-finger transcription factor neuron-derived orphan receptor (NOR-1) in the retina is a novel finding. The downregulation of epidermal growth factor (EGF) which is expressed by retinal second- and third-order neurons in the retina,[29] may be associated with the upregulation of the EGF receptor (Table 2), and implicated in the neuronal remodeling in the detached inner retina.[5] The downregulation of arrestin-C suggests a functional impairment of cones after detachment. The downregulation of blue-sensitive opsin (Table 4) suggests a reduction in phototransduction especially in blue cones, and reflects the well-known blue–yellow color confusion after rhegmatogenous retinal detachment in human subjects.

Most of the identified upregulated genes in the detached retina (52 of 85 genes) and in the peridetached retina (13 of 28 genes) represent genes that are involved in inflammation and immune responses (Tables 1, 2). The gene with the highest increase in the detached retina is the gene for lysozyme (Table 2). Lysozyme is a soluble mediator of the innate immune

defense, which also has important functions in intracellular proteolysis and protection against oxidative stress. In addition to lysozyme, various other soluble mediators of the innate immune defense, including complement proteins and lactoferrin (Table 3) were activated after detachment. We found that lysozyme is constitutively expressed in the porcine retina, whereas complement proteins and lactoferrin are induced after detachment. Another inflammatory mediator strongly activated in detached and peridetached retinas is MCP-1 (Table 2), which has been described to be increasingly expressed by Müller cells after detachment of the rat retina.[20] In the detached retina, at least 15 of the inflammation-related genes represent interferon-stimulated genes, for example major histocompatibility (MHC) class I and II molecules, interferon regulatory factors, double stranded RNA-dependent protein kinase, Mx protein, Toll-like receptor-2 and -4, and NADPH oxidase. One of the factors that may induce an increase in the level of interferon is interleukin-18 (Table 2), a proinflammatory cytokine which is converted to a biologically active molecule by interleukin-1β converting enzyme (caspase 1; Table 3), which is also a marker gene of photoreceptor apoptosis.[30]

A second group of genes activated after detachment are genes for proteins involved in intracellular protein degradation. This group includes proteins of the ubiquitin-proteasome system (ubiquitin-specific protease, PSMD4, PSME1 and -2, and LMP2) which plays a central role in cellular homeostasis and in regulation of inflammatory and stress responses, as well as marker genes for lysosomal protease activity and autophagy (lysozyme, cathepsin H).

A third group of genes activated after detachment are genes for antioxidant proteins and proteins involved in metal homeostasis. Upregulated genes for antioxidant proteins include metallothioneins (Tables 2 and 3), thioltransferase, manganese superoxide dismutase, cytosolic glutathione peroxidase (Table 2), and lactoferrin (Table 3). In addition to the cupric and ferrireductase STEAP (Table 2), some of the antioxidant proteins are implicated in metal homeostasis. The data suggest that oxidative stress, which may affect the metal homeostasis in the retina, is one major factor that triggers cellular responses in both the detached and peridetached retina. Upregulation of the NADPH oxidase (Table 3), a component of the phagocyte superoxide anion-generating system, may contribute to the oxidative stress in the detached retina.

We found that four coagulation/fibrinolysis-related proteins are upregulated in the detached retina: plasminogen activator and its inhibitor, nexin-1, and tissue factor (Table 2). These factors are implicated in the regulation of blood coagulation, as well as in the remodeling of the extracellular matrix. The upregulation of genes for fibronectin and tissue inhibitor of metalloproteinase (TIMP)-1 (Table 2) suggests that extracellular matrix remodeling is enhanced in both detached and peridetached retinas. Extracellular matrix remodeling in the detached retina is also indicated by the upregulation of connective tissue growth factor (CTGF; Table 2), the downregulation of fibromodulin (Table 4), and the upregulation of GP38K (Table 3), which is a secreted glycoprotein highly expressed in regions of tissue remodeling. Moreover, the expression of ameloblastin (Table 3), until now only recognized as a structural protein of the enamel matrix, suggests enhanced extracellular matrix remodeling after detachment.

Real-time PCR analysis validated the data of the microarray analysis (Tables 2, 3). Apparently, the sensitivity of the real-time PCR analysis of the neural retina was higher than the sensitivity of the microarray analysis. The real-time PCR analysis revealed also that the pigment epithelium underlying the detached and peridetached retinas in situ, as well as Müller cells, displayed similar alterations in the expression of selected genes as the whole neural retina (Tables 2, 3). The data obtained in Müller cell–enriched preparations may suggest that Müller cells are one but not the only source of the elevated expression of the most genes investigated. Apparently, multiple cell types are involved in the response of the neural retina to detachment.

The structural alterations in the retinal tissue were examined in semithin sections (Fig. 1) and by electron microscopy (Fig. 2). Neither the photoreceptor cell layer nor the photoreceptor–pigment epithelium interface displayed any obvious structural alterations in peridetached retinas compared with the control (Figs. 1, 2A–C). In detached retinas, there were areas with strongly disrupted or disorganized photoreceptors (Fig. 1), as well as areas where photoreceptor degeneration was less pronounced (Fig. 2D). In the latter areas, cones were either structurally intact (Fig. 2D) or fully disrupted (not shown), whereas the rods were preserved but showed structural alterations. These alterations included swelling and disruption of mitochondria (Figs. 2D–H) and the presence of vesicle-like structures (Fig. 2D–G) and stacks of outer segment membranes within the inner segments (Figs. 2E, 2F). Several degenerating neuronal cell bodies with mitochondrial swelling and intracellular edema were found in the ganglion cell (Fig. 2I) and inner nuclear layers (Fig. 2L) of detached retinas. Cystoid spaces (indicating extracellular edema) were present in the outer nuclear, inner nuclear, and ganglion cell layers of detached retinas (arrows in Figs. 1, 2J, 2K, 2M). There were accumulations of putative microglial cells in the nerve fiber and ganglion cell layers of detached and peridetached retinas (Fig. 1, arrowheads) which was not observed in control tissues. Likewise, the inner nuclear layer of detached retinas contained putative microglial cells (Fig. 2L) which was not observed in control tissues. Polymorphonuclear leukocytes accumulated within the vessels of detached and peridetached retinas, whereas the vessels in control tissues contained predominantly erythrocytes (Fig. 1). Apparently, the walls of retinal vessels were not leaky in all tissue samples investigated. In peridetached tissues, leukocyte accumulation was observed only within the retinal vessels, but not in the choroidal vessels. The data suggest that degeneration of photoreceptors and inner retinal neurons, as well as the formation of extracellular edema, were largely restricted to the detached retina, whereas activation of immune cells was observed in both the detached and the peridetached retinal areas.

Discussion

The present study provides an overview of early gene expression alterations in the porcine retina after experimental detachment. The alterations in gene expression indicate that retinal detachment induces a tripartite response in the retina, consisting of inflammation, immune responses, and coagulation/fibrinolysis. This process is predominantly mediated by activation of microglial cells in the inner retinal tissue, and by accumulation of leukocytes within the retinal vessels. The data confirm previous studies suggesting that oxidative stress (likely resulting from outer retinal hypoxia and activation of immune cells) is a major factor that triggers cellular responses in the detached retina. The gene expression alterations are not restricted to the detached retinal areas but are also observed in the nondetached retina surrounding local detachment in situ, as well as in the underlying pigment epithelium.

Intracellular Proteolysis

We found that one group of upregulated genes represent genes for factors involved in intracellular protein degradation. After detachment, intracellular proteolysis may be involved in various cellular processes such as phagocytosis of debris, antigen

TABLE 2. Upregulated Genes in Detached and Peridetached Retinas

Accession Number	Gene	Increase in Expression	
		Detached	Peridetached
Immune Response and Inflammation-Related			
NM_214392	Lysozyme (LYZ)	158.9 ± 87.1	99.9 ± 61.2
		427.6 ± 233.2	*108.9 ± 17.5*
		(52.5 ± 18.7)	(35.5 ± 14.0)
		[14.0 ± 6.7]	[22.1 ± 4.7]
NM_214214	Monocyte chemoattractant protein 1 (MCP-1)	53.4 ± 5.7	28.3 ± 14.7
		69.3 ± 36.4	*32.5 ± 6.0*
		(51.4 ± 13.8)	(41.2 ± 8.1)
		[35.2 ± 14.8]	[19.1 ± 10.9]
NM_213804	RNA helicase (RHIV-1)	14.4 ± 4.3	
NM_214262	Interleukin 1 receptor antagonist (IL1RN)	12.5 ± 4.0	
AY609555	Annexin I (ANXA1)	11.8 ± 0.5	4.2 ± 1.7
		15.0 ± 6.5	*7.7 ± 5.1*
		(3.7 ± 1.5)	(3.1 ± 0.7)
		[2.9 ± 0.6]	[2.8 ± 1.3]
NM_214303	2′,5′ Oligoadenylate synthetase (OAS1)	11.6 ± 5.8	
AY285933	MHC class II antigen (SLA-DRA1)	11.3 ± 7.0	
		14.8 ± 6.8	*10.5 ± 5.8*
		(3.4 ± 0.6)	(7.3 ± 1.7)
		[1.2 ± 0.7]	[1.5 ± 0.6]
AB004800	S100C Protein (S100A11)	11.2 ± 1.9	5.7 ± 2.5
AY706383	Annexin II (ANXA2)	9.3 ± 1.8	4.4 ± 0.8
AJ583706	Interferon regulatory factor 1 (IRF1)	8.3 ± 2.7	4.0 ± 1.9
NM_214061	Mx protein (MX2)	7.6 ± 2.0	
AY450287	Interleukin-18 (IL18)	5.6 ± 1.7	
BI118294	IFNAR1 gene, IFNGR2 gene	4.8 ± 1.5	
NM_001001861	Chemokine ligand 2 (CXCL2)	4.6 ± 2.1	
NM_214319	Double-stranded RNA-dependent protein kinase (PKR)	4.1 ± 0.6	
AY135590	Nonfunctional MHC class I antigen (SLA-1)	3.2 ± 0.3	
NM_21389	Vascular cell adhesion molecule (VCAM)	3.2 ± 1.0	
NM_213969	p55 TNF receptor (TNFRSF1A)	3.1 ± 1.3	
NM_213978	β2-Microglobulin (B2M)	3.1 ± 0.2	
NM_213773	Chemokine (C-X-C motif) receptor 4 (CXCR4)	3.1 ± 0.7	
Antioxidants and Metal Homeostasis-Related			
AB000794	Metallothionein isoform (MT-2B)	12.8 ± 1.9	9.1 ± 3.5
		44.9 ± 23.7	*52.1 ± 24.9*
		(1.5 ± 0.6)	(3.3 ± 1.0)
		[17.4 ± 1.3]	[9.4 ± 1.8]
NM_214233	Thioltransferase (GLRX1)	8.1 ± 2.9	3.1 ± 1.1
NM_214127	Manganese superoxide dismutase (SOD2)	7.2 ± 3.0	
NM_214201	Cytosolic glutathione peroxidase (GPX1)	4.7 ± 2.7	
NM_214305	Six transmembrane endothelial antigen of PAEC (STEAP)	3.7 ± 1.0	
Intracellular Proteolysis			
NM_213826	Ubiquitin-specific protease (UBP)	8.8 ± 0.9	
CK461474	Proteasome 26S subunit non-ATPase 4 (PSMD4)	6.1 ± 2.7	5.8 ± 0.8
NM_214279	Proteasome activator 28 beta subunit (PSME2)	6.0 ± 0.9	
NM_214304	Proteasome activator 28 alpha subunit (PSME1)	4.8 ± 0.7	
Blood Coagulation-Related			
NM_213910	Plasminogen activator inhibitor I (PLANH1)	7.8 ± 2.6	
NM_213945	Plasminogen activator (PLAU)	6.6 ± 1.7	
NM_214287	Nexin-1 (PN-1)	3.6 ± 0.9	3.9 ± 2.6
NM_213785	Tissue factor (LOC396677)	3.6 ± 0.4	
		2.6 ± 0.9	*2.6 ± 1.2*
		(2.9 ± 0.8)	(2.5 ± 0.8)
		[2.0 ± 0.2]	[2.2 ± 1.4]
Signaling and Nuclear Proteins			
NM_213769	Signal transducer and activator of transcription 1 (STAT1)	4.9 ± 0.8	
Transport Proteins			
NM_214076	L-Kynurenine 3-mono-oxygenase Fpk (KMO)	3.1 ± 0.8	

(*continues*)

TABLE 2 (*continued*). Upregulated Genes in Detached and Peridetached Retinas

Accession Number	Gene	Increase in Expression	
		Detached	Peridetached
Extracellular Matrix			
AY274117	Fibronectin (FN1)	15.5 ± 7.5	5.8 ± 2.0
NM_213857	Metalloproteinase tissue inhibitor 1 (TIMP1)	11.6 ± 3.2	6.7 ± 4.1
Secretory Proteins			
AY669080	Bone morphogenetic protein 2 (BMP2)	19.1 ± 4.5 *211.1 ± 86.5* (7.0 ± 2.0) [18.6 ± 5.9]	5.1 ± 0.5 *22.8 ± 10.7* (3.3 ± 0.9) [2.8 ± 0.9]
AY509877	Alpha-2-macroglobulin (A2M)	7.9 ± 1.7	3.1 ± 0.9
BI181686	Connective tissue growth factor (CTGF)	3.6 ± 2.5	
Membrane Proteins			
BX926326	Epidermal growth factor receptor (EGFR)	16.9 ± 4.8	
BE030517	MOESIN protein (MOESIN)	4.3 ± 2.0	
Intracellular Proteins			
NM_001039746	Fatty acid binding protein 5 (FABP5)	5.5 ± 1.0	4.8 ± 1.9
AY533020	Cellular FLICE-like inhibitory protein (C-FLIP)	4.2 ± 1.3	
NM_214181	Putative inhibitor of apoptosis (PIAP)	3.9 ± 0.9	

Identified genes are listed that showed more than a threefold change in expression level and were therefore considered to be upregulated in detached or peridetached neural retinas compared with control retinas. Four detached and peridetached retinas were investigated, and the magnitude of change for a specific gene was estimated by using the mean value of control retinas. Data obtained with real-time PCR in neural retinas are shown italic. Data obtained with real-time PCR in pigment epithelial cells are shown in parentheses, and data obtained with real-time PCR in Müller cell-enriched cell preparations are shown in brackets.

processing, photoreceptor apoptosis, and adaptation of the cells to an abrupt change in the metabolic conditions. Retinal detachment causes metabolic stress in the photoreceptor cell layer, due to the prolongation of the diffusion pathway for oxygen and nutrients from the choroid and the disruption of the regeneration cycle of visual pigments. Metabolic stress in photoreceptor cells can be balanced by an autophagic decrease in the level of phototransduction proteins.[31,32] The decrease in the photoreceptor activity may protect against cell death.[32] There are ultrastructural alterations that may suggest an autophagic deconstruction of photoreceptor segments in the detached retina: the presence of stacks of outer segment membranes (Figs. 2E, 2F) and vesicle-like structures (Figs. 2D–G) in the rod inner segments. However, the nature of the vesicle-like structures remains to be determined. We assume that these structures represent lipid droplets arising from autophagy of outer segment membranes and degenerated mitochondria.

Overstimulation of proteolytic pathways may be also more directly involved in the execution of or the protection against photoreceptor apoptosis. Retinal detachment is associated with a distinct level of photoreceptor apoptosis.[7] Apoptosis is caspase dependent or independent (performed by proteolytic enzymes such as cathepsins). It has been shown that intracellular protein degradation in autophagosomes, lysosomes, and proteasomes operate either independently or in concert with caspases to initiate and execute apoptosis.[33] Abundant autophagic vacuoles in rod inner segments were observed in early stages of light-induced retinal degeneration, suggesting a possible link between autophagy and photoreceptor apoptosis.[33] In neurons, autophagy can be activated by apoptosis signals.[34] Oxidative stress is one factor that triggers cellular responses to detachment.[16] Oxidized proteins in the retina can be removed by proteasomes, and the removal of these proteins may decrease the effect of oxidative stress on apoptosis.[35,36] Other mechanisms to protect against apoptosis involve the upregulation of various antiapoptotic genes—for example, leukemia inhibitory factor (Table 3), the putative inhibitor of apoptosis (PIAP; Table 2), which is known to prevent programmed cell death during inflammation, and the cellular FLICE-like inhibitory protein (Table 2), which prevents Fas- and tumor necrosis factor–mediated apoptosis.

Cellular Immune Responses

The upregulation of inflammation- and immune response-related genes after detachment may have three causes: activation of microglial and other immune-competent cells of the retina, invasion of monocytes/macrophages into the subretinal space, and adhesion of leukocytes to vascular endothelial cells. It is known that photoreceptor-specific proteins are potent immunogens.[37] Retinal detachment may cause a release of proteins from disrupted photoreceptor segments, resulting in inflammation and immune responses. After detachment, monocytes/macrophages (which express CD163 and urate transporter/channel protein, for example; Table 3) invade the subretinal space and phagocytize disrupted outer segments. Microglia are the primary antigen-presenting cells in the retina. Retinal detachment causes strong proliferation and migration of microglial cells from the innermost retinal layers to the outer retina.[17,19] In a recent study, we found an increase in the number of microglial cells at the inner surface of detached and peridetached retinas of the pig of ~400% and ~200%, respectively.[19] Since these cells did not change their ramified morphology,[19] it was concluded that the immune cell response in the parenchyma of detached and peridetached retinas is mediated predominantly by activation of resident microglial cells, whereas extravasation of (nonramified) immune cells is marginal. Microglial cells play important roles in host defense against invading microorganisms and in immunoregulation and tissue repair. They participate in the phagocytosis of debris and facilitate regenerative processes. Retinal microglia express MHC class I and II molecules,[38] for example, which mediate antigen presentation after processing by proteases of the en-

TABLE 3. Genes Only Detectable in Detached and Peridetached Retinas

Accession Number	Gene Description	Increase in Expression	
		Detached	Peridetached
Immune Response and Inflammation-Related			
M81771	Ig gamma 2b chain constant region (IgG2b)	82.8 ± 64.9	
M92050	Ig mu-chain	79.4 ± 62.9	
NM_213761	Toll-like receptor 2 (TLR2)	55.0 ± 9.4	
		16.2 ± 4.9	*20.7 ± 10.3*
		(6.8 ± 4.2)	(3.5 ± 0.9)
NM_214048	Arginase I (ARG1)	48.4 ± 17.1	7.9 ± 4.2
NM_213828	IgG heavy chain (LOC396781)	43.1 ± 52.7	8.9 ± 8.2
U12594	Immunoglobulin alpha heavy chain constant region (IgA C alpha)	21.7 ± 10.7	
AY349421	Complement C1r (C1R)	20.5 ± 4.8	16.9 ± 2.5
NM_213976	Putative CD163 antigen (CD163)	16.4 ± 8.2	14.4 ± 2.4
AB188301	Toll-like receptor 4 (TLR4)	15.2 ± 3.4	
		15.7 ± 6.0	*8.0 ± 4.6*
		(6.7 ± 1.6)	(5.1 ± 2.0)
		[3.6 ± 1.4]	[8.6 ± 0.9]
M59322	Ig rearranged lambda chain	14.7 ± 2.5	8.2 ± 6.0
NM_214402	Leukemia inhibitory factor (LIF)	12.9 ± 8.0	
NM_001001621	Chemokine C-C motif receptor 1 (CCR1)	12.4 ± 3.1	
M59321	Ig rearranged kappa chain V-J-C-regions	10.9 ± 5.4	
NM_213867	Alveolar macro-phage-derived chemotactic factor-I (AMCF-I)	10.9 ± 9.2	
AY349426	Complement component C1s (C1S)	10.6 ± 2.8	8.3 ± 4.1
NM_213771	Interleukin 10 receptor β (IL10RB)	10.6 ± 2.9	6.9 ± 4.0
NM_213932	Urate transporter/ channel protein (UATP.I)	10.4 ± 1.8	
CK450954	Histone H1.2-like protein (LOC574051)	9.6 ± 5.2	
NM_214202	Immunoreceptor DAP12 (TYROBP)	7.5 ± 2.3	
AF299325	Allograft inflammatory factor-1 (AIF1)	7.3 ± 2.7	3.8 ± 1.2
		9.5 ± 5.4	*9.1 ± 6.0*
		(4.5 ± 2.8)	(2.4 ± 1.4)
		[2.8 ± 0.4]	[6.9 ± 2.9]
AF103945	CCAAT/enhancer binding protein beta (CEBPB)	7.2 ± 1.0	5.2 ± 0.4
NM_213908	CD18 leukocyte adhesion molecule (CD18)	6.8 ± 3.1	
NM_214043	NADPH oxidase heavy chain subunit (GP91-PHOX - electron transport)	6.0 ± 1.9	
NM_213831	CD1 antigen (CD1.1)	5.7 ± 3.6	
AF372455	FcgammaRIII a.1 (FCGR3B)	5.5 ± 0.2	
AY459300	SLA-DQ beta1 domain (SLA-DQB1)	5.3 ± 1.7	
CF789161	Interferon regulatory factor 7 (IRF7)	5.1 ± 2.1	
NM_001033013	Fc gamma receptor II (FCGR2B, CD32)	4.8 ± 0.5	
AB032169	SLA-DM alpha chain (SLA-DMA)	4.3 ± 1.5	
NM_214162	Interleukin-1β converting enzyme (CASP1)	4.2 ± 0.7	
AF148221	Fc epsilon receptor gamma chain (FCER1G)	4.0 ± 1.5	
NM_213811	Scavenger receptor for phosphatidyl-serine and oxidized low density lipoprotein (SR-PSOX)	3.2 ± 0.2	
Antioxidants and Metal Homeostasis-Related			
NM_001001266	Metallothionein (MT1A)	107.1 ± 14.9	132.5 ± 43.5
		16.9 ± 6.4	*22.7 ± 8.9*
		(17.2 ± 7.2)	(12.7 ± 3.0)
		[55.9 ± 26.2]	[56.8 ± 33.1]
M81327	Lactoferrin (PLF)	11.0 ± 6.8	
Intracellular Proteolysis			
NM_001037961	Proteasome beta 9 subunit (LMP2)	67.6 ± 10.3	17.7 ± 8.1
NM_213929	Cathepsin H (CTSH)	8.6 ± 2.5	
Secretory Proteins			
NM_214037	Ameloblastin (AMBN)	11.5 ± 4.1	
		22.9 ± 10.7	*15.1 ± 10.6*
		(2.3 ± 0.8)	(2.1 ± 0.5)
		[3.4 ± 2.2]	[7.0 ± 0.1]
NM_001001540	38-kDa heparin-binding glycoprotein (GP38K)	26.4 ± 8.7	
Intracellular Proteins			
AJ686718	Polypeptide chain elongation factor 1 alpha (EF1A1)	15.4 ± 6.0	10.3 ± 2.5
NM_213810	Alpha-1,3-galactosyltransferase (GGTA1)	4.9 ± 1.4	3.7 ± 1.5

Identified genes are listed that were expressed in detached and peridetached neural retinas but were classified as absent in control retinas. Four detached and peridetached retinas were investigated, and the magnitude of change was estimated by using the mean value of control retinas. Each mean value from the control retinas was nonzero but was so low that it could not be classified as expressed. Data obtained with real-time PCR in neural retinas are shown italic. Data obtained with real-time PCR in pigment epithelial cells are shown in parentheses, and data obtained with real-time PCR in Müller cell-enriched cell preparations are shown in brackets.

TABLE 4. Downregulated and Nondetectable Genes in Detached Retinas

Accession Number	Gene Description	Decrease in Expression
Downregulated Genes		
NM_214247	Neuron-derived orphan receptor-1 alfa (NOR-1)	−6.6 ± 2.8
AF159383	Fibromodulin (FMOD)	−6.1 ± 1.1
NM_214345	Arrestin-C (ARR3)	−4.2 ± 1.9
NM_214020	Epidermal growth factor (EGF)	−3.4 ± 1.3
Nondetectable Genes		
NM_214099	Insulin-like growth factor binding protein-5 (IGFBP-5)	
NM_214090	Blue-sensitive opsin (OPN1SW)	
NM_001008688	Carbonic anhydrase III (CA3)	

Identified genes are listed that were expressed in control retinas but were decreased or classified as absent in detached neural retinas.

dosomal–lysosomal system. It has been shown in the rabbit retina that the perivascular microglia increase the expression of CD18 and MHC class II under inflammatory conditions and are associated with deposits of fibrin.[39] In addition to microglia, inflammatory and immune responses may be mediated by other retinal cell types, for example Müller cells (which phagocytose debris and express MCP-1 after detachment)[20,40] and astrocytes. Astrocytes and Müller cells express MHC class II molecules under distinct conditions (e.g., in response to oxidative stress[41]). Müller cells can also express Fc gamma receptors.[42] In the present study, Müller cells displayed an activation of some inflammation-related genes such as lysozyme and MCP-1 after detachment, whereas MHC class II was not upregulated (Table 2).

In addition to the activation of micro- and macroglial cells, an increased adhesion of leukocytes to endothelial cells of the retinal vessels (mediated by the cell adhesion molecules VCAM and CD18; Tables 2, 3) is likely to contribute to the upregulation of immune response-related genes found in the present study. We observed an accumulation of polymorphonuclear leukocytes within the vessels of detached and peridetached retinas but not of control retinas. Retinal leukostasis may be a causative factor in the decrease in the retinal blood flow rate observed in human subjects with retinal detachment.[10,11] Leukocytes adhering to the vascular endothelium may also be a source of oxidative–nitrosative stress in the inner retina.[43] We found a similar activation of distinct inflammation- and immune response–related genes in the pigment epithelium (Tables 2, 3). It is known that retinal pigment epithelial cells act as antigen-presenting cells and may express MHC class I and II molecules after stimulation with interferon, for example.[44]

Inflammatory Mechanisms

Various immune response factors may also be involved in other cellular responses such as photoreceptor cell death, defense against oxidative stress, and adaptation to altered metabolic conditions. There are multiple death pathways involved in photoreceptor degeneration—for example, apoptosis, autophagy, neuroinflammation, and complement-mediated lysis.[30] Lysozyme, which is the factor with the strongest upregulation in the detached retina (Table 2), is a key marker of lysosomal protease activity and autophagy implicated in photoreceptor cell death.[30] In addition to its proapoptotic role, lysozyme has many other functions, including a role in surveillance of cellular membranes, stimulation of proliferation and phagocytic activity of immune cells, and negative regulation of inflammation. Lysozyme is also an antioxidant protein, and suppresses oxidant-induced apoptosis.[45] We found an early activation of genes for complement components in the detached and peridetached retinal tissue (Table 3). It has been shown in mouse

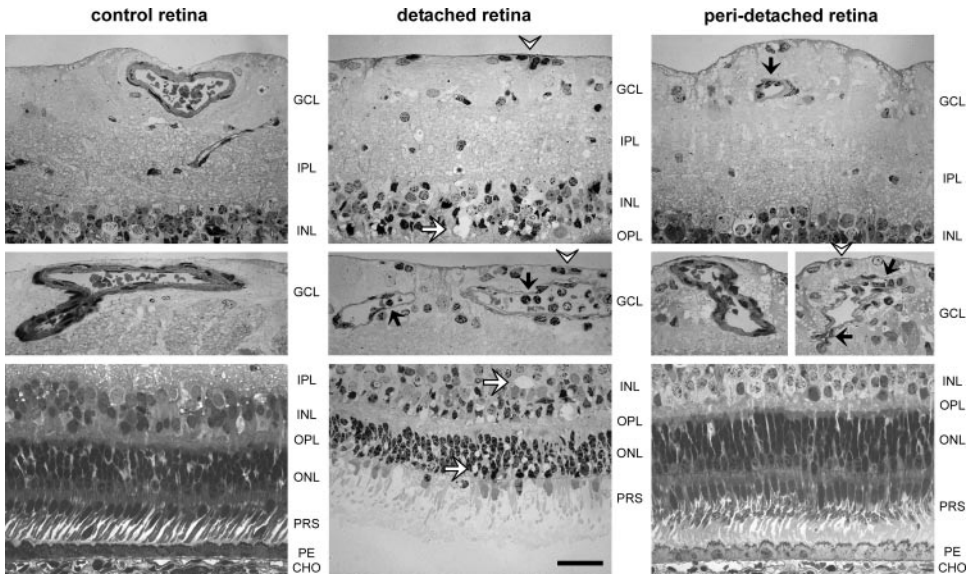

FIGURE 1. Experimental detachment caused structural changes in the porcine retina. The detached retina displayed disruption of the photoreceptor segments (PRS) not observed in the peridetached and control retinas. The outer (ONL) and inner (INL) nuclear layers contained cystoid spaces (*white arrows*) reflecting extracellular edema. Accumulations of putative microglial cells (*arrowheads)* were present in the inner retinal layers of detached and peridetached retinas but not in the control tissue. The vessels of the control retina contained predominantly erythrocytes, whereas the vessels of the detached and peridetached retina contained accumulations of leukocytes (*black arrows*). The images were taken from semithin retinal sections. CHO, choroidea; GCL, ganglion cell layer; IPL, inner plexiform layer; OPL, outer plexiform layer; PE, pigment epithelium; Bar, 30 μm (for all images).

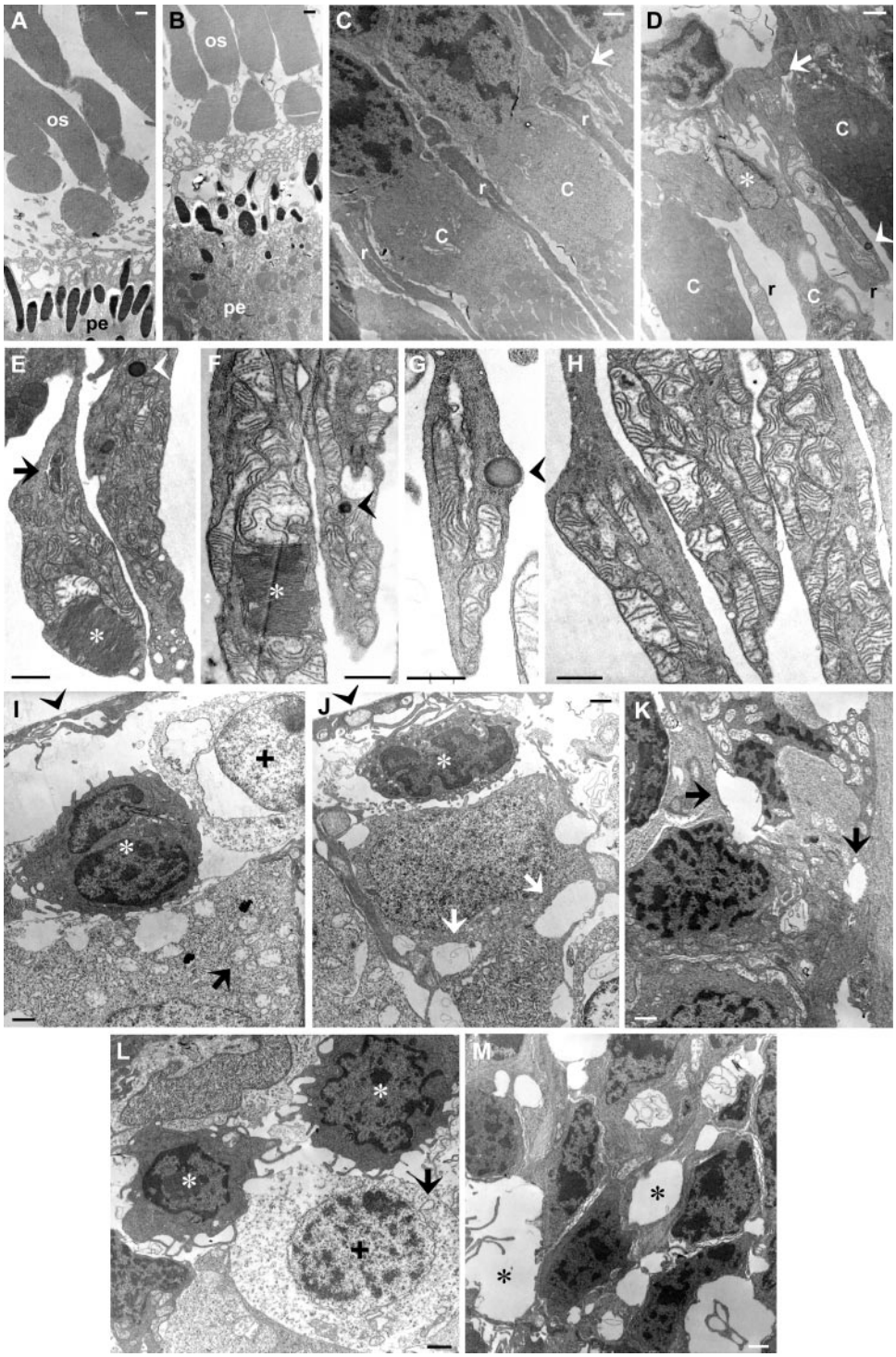

FIGURE 2. Ultrastructural changes in the porcine retina after experimental detachment. Electron microscopic images were taken from control (**A**), peridetached (**B**, **C**), and detached retinal areas (**D**–**M**). (**A**, **B**) The interface between photoreceptor outer segments (os) and the pigment epithelium (pe) showed no structural alteration in a peridetached retina (**B**) compared with a control retina (**A**). (**C**) Regular photoreceptor morphology in a peridetached retina. *Arrow*: outer limiting membrane; r, rod; c, cone. (**D**) In a detached retina, a rod inner segment displayed mitochondrial swelling and contained a large vesicle (*arrowhead*). A photoreceptor cell nucleus (✻) had migrated through the outer limiting membrane (*arrow*). (**E**–**G**) Rod inner segments contain vesicles (*arrowheads*), stacks of outer segment membranes (✻), and swollen mitochondria. *Arrow*: a putative autophagic vacuole. (**H**) Rod inner segments with swollen and disrupted mitochondria that contained expanded cristae. (**I**) A cell body in the ganglion cell layer of a detached retina displayed intracellular edema (+), whereas another cell body contained swollen mitochondria (*arrow*). (✻) Putative microglial cell. (**J**) The ganglion cell layer of a detached retina contained edematous cystoid spaces (*arrows*) and a putative microglial cell (✻). *Arrowhead*: inner limiting membrane. (**K**, **L**) The inner nuclear layer of a detached retina contained cystoid spaces (*arrows*), microglial cells (✻), and several degenerating cells, displaying intracellular edema (+) associated with an enlargement of the perinuclear space (*arrow*). (**M**) Cystoid spaces (✻) in the ONL of a detached retina. Bars, 1 μm.

models of inherited and light-induced photoreceptor degeneration that complement activation precedes apoptosis and autophagy.[30] In addition to the lysis of cells and enhancement of apoptosis, the activated complement system was shown to be involved in the recruitment of inflammatory cells, in the generation of proinflammatory, prothrombotic, and profibrotic mediators, and in the clearance of apoptotic cells. Lactoferrin (Table 3), another soluble mediator of the innate immune defense, is a stimulator of the local immune response. It inhibits the activity of proinflammatory cytokines, is a transporter for metal ions such as iron and zinc, and protects against the toxicity of reactive oxygen and iron radicals. Toll-like receptors (Table 3) have functional roles in initiating innate immune and inflammatory responses and in the activation of antigen-presenting cells; however, they are also involved in transmembrane signaling in response to endogenous factors. Toll-like receptor-4 is implicated in the transmembrane signaling that contributes to the management of outer segments by pigment epithelial cells. This action is mediated by metabolic and calcium signals and by the production of oxidants.[46] RNA helicases (Table 2) are implicated in antiviral responses but also in any cellular process involving RNA metabolism such as tran-

scription, splicing, and translation. In addition to Toll-like receptors, RNA helicases induce the expression of type I interferon and inflammatory cytokines. The anti-inflammatory protein, annexin I (Table 2), which forms complexes with S100C (Table 2), promotes caspase-3-mediated inflammatory cell apoptosis, and is the "eat-me" signal on apoptotic cells to be recognized by phagocytes. The upregulation of at least 15 interferon-stimulated genes suggests the presence of elevated interferon levels after detachment. In addition to its essential role in antiviral immunity, the interferon system has a wide range of biological functions regarding the regulation of cell metabolism, growth control, and apoptosis. There are various interferon-stimulated genes with proapoptotic functions such as 2′-5′ oligoadenylate synthetase (Table 2) and interferon regulatory factors (Tables 2, 3). Several interferon-induced transcripts can play a protective role in preventing immune cell infiltration, and the presence of interferon in the eye can reduce damaging inflammatory responses.[47] A similar upregulation of interferon-stimulated and inflammation-related genes has been described in the light-damaged mouse retina.[48]

Blood Coagulation/Fibrinolysis

Commonly, blood coagulation, immune responses, and inflammation are coupled. We found that different blood coagulation and fibrinolysis–related proteins are upregulated in the detached retina (Table 2). Activation of the blood coagulation cascade may contribute to the decline in the retinal blood flow observed in patients with retinal detachment.[10,11] Since various coagulant mediators (e.g., factor Xa and fibrin) are proinflammatory, upregulation of the tissue factor (Table 2) may promote inflammation through initiation of the coagulation cascade. Plasminogen activator and its inhibitor (Table 2) regulate the balance between coagulation and fibrinolysis. The protease nexin-1 (Table 2) inhibits the activity of thrombin, plasmin, and plasminogen activators. In addition, annexin II (Table 2) serves as a profibrinolytic co-receptor for plasminogen and tissue plasminogen activator—for example, on the surface of vascular endothelial cells. Annexin II is involved in endothelial cell activation, and induces the expression of cell adhesion molecules such as VCAM (Table 2). However, in addition to blood coagulation, some of these proteins also have important roles in tissue remodeling and neuronal cell death and survival. Tissue plasminogen activator expressed by, for example, retinal glial and ganglion cells facilitates excitotoxic cell death in the retina.[49,50] Plasminogen activator and its inhibitor are implicated in remodeling of extracellular matrix and synaptic plasticity. Nexin-1 is the glia-derived neurite-promoting factor that, via extracellular proteolytic activity, enhances the survival and growth of neurons and inhibits cellular adhesion and migration. Annexin II is involved in plasmin-mediated dissolution of the extracellular matrix. It remains to be determined whether the migration of microglial cells[19] and the remodeling of glial and neuronal cells observed after experimental detachment[6] are supported by these proteins. Neuronal remodeling after detachment, such as neurite outgrowth from bipolar and horizontal cells,[6] may also be facilitated by bone morphogenetic protein-2 (Table 2) which promotes neurite extension.

Other/Novel Factors

We found an upregulation of the kynurenine 3-mono-oxygenase in the detached retina (Table 2). Activation of the kynurenine pathway, resulting in an accumulation of the excitotoxin quinolinic acid, occurs in several inflammatory disorders of the central nervous system.[51] Kynurenine metabolites cause apoptotic or necrotic neuronal cell death in vitro, and inhibition of the kynurenine 3-mono-oxygenase reduces neuronal death in culture and animal models of brain ischemia or excitotoxicity.[51] Excitotoxicity is suggested to represent one factor underlying the morphologic and biochemical alterations in the inner retina after detachment.[6,52]

There are at least two genes which we describe for the first time to be expressed in the retina, ameloblastin (Table 3) and the neuron-derived orphan receptor (NOR-1) (Table 4). NOR-1 is a transcription factor implicated in oncogenesis, apoptosis, proliferation of vascular smooth muscle cells, and neuroprotection—for example, after cerebral ischemia.[53] NOR-1-knockout mice display impaired hippocampal neuronal survival and axon guidance.[54] Whether the downregulation of NOR-1 is implicated in photoreceptor apoptosis, neuroprotection, or neuronal remodeling after detachment remains to be determined.

Involvement of Nondetached Adjacent Retinal Areas

After local retinal detachment, reactive changes are not restricted to the detached retina but are also observed in the surrounding nondetached tissue. In human subjects, functional defects were observed in areas of the visual field that correspond to detached and nondetached retinal regions.[1,21,22] The retinal blow flow rate is decreased (albeit less than in the detached retina) also in the peridetached retina.[10] In animal models of local retinal detachment, degeneration of photoreceptor cells, Müller cell gliosis, and activation of microglial cells were observed in both detached and peridetached retinal tissues.[12,18,19,26,27] These alterations had lower incidence and amplitude and occurred after longer periods, when compared with the changes in the detached retina. Herein, we report that alterations in the gene expression occurred early after experimental detachment in both the detached and peridetached retinas of the pig. We found that the alterations in gene expression in peridetached retinas involved genes implicated in inflammation and immune responses, response to oxidative stress, and intracellular proteolysis. The alterations in gene expression in peridetached retinas were less pronounced than in detached retinal areas, both in the number of genes and in the amplitude of expression alteration. Whereas the degeneration of photoreceptors and inner retinal neurons and the formation of extracellular edema were largely restricted to the detached retina, immune cells accumulated in both the detached and peridetached tissues. We assume that microglia activation and leukostasis observed in the peridetached retinas (Fig. 1) are caused by inflammatory factors that diffuse from the detached into the surrounding tissue. Since the photoreceptors in peridetached retinas did not show structural alterations (Figs. 1, 2B, 2C), we suggest that the early response of immune cells in the inner retina underlies the gene expression alterations in peridetached areas.

Müller Cell Gliosis

Inflammation and immune responses in the detached and peridetached retinas may be also causative factors for the induction of Müller cell gliosis. A major response of Müller cells on retinal detachment is a decrease in the expression of potassium channels and an induction of cellular swelling, suggesting a disturbance of Müller cell–mediated retinal ion and water homeostasis.[12,13,18,19] Inflammatory lipid mediators such as arachidonic acid and prostaglandins, as well as oxidative stress, have been causally implicated in the alteration of Müller cell swelling characteristics after detachment.[18] An impairment of the homeostatic functions of Müller cells may contribute to the death of photoreceptor cells observed in the nondetached retina surrounding local detachment.[26,27] A contribution of Müller cells to the degeneration of photoreceptor cells after detach-

ment is also suggested by the recent finding that MCP-1 (which is increasingly expressed by Müller cells after detachment)[20] promotes photoreceptor apoptosis, probably due to its ability to recruit neurotoxic phagocytotic monocytes to the injured area.[55]

Conclusions

In summary, the present study confirms, for the first time at the gene expression level, that experimental local detachment causes alterations not only in the detached retina but also in the surrounding nondetached retina. This may explain observations in human subjects showing that, in cases of local detachment, the vision loss[1,21,22] and the decrease in retinal blood flow[10] are not restricted to the detached retina but are also observed in the surrounding nondetached tissue. The present data suggest that one causative factor of functional alterations after detachment is the early activation of a tripartite process involving inflammation, immune responses, and coagulation/fibrinolysis, which is probably triggered by the deconstruction of photoreceptors and mediated by activation of immune cells in the inner retina. Since inflammatory mediators are suggested to increase the rate of photoreceptor cell death in the detached retina,[20,55] blockage of the early detachment-induced inflammatory response—for example, by the use of anti-inflammatory agents in the course of reattachment surgery—may have therapeutic benefit for patients with retinal detachment.

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