Caspase-1 Ablation Protects Photoreceptors in a Model of Autosomal Dominant Retinitis Pigmentosa

Marijana Samardzija, Andreas Wenzel, Markus Thiersch, Rico Frigg, Charlotte Remé, and Christian Grimm

**PURPOSE.** Caspase-1 gene expression has been reported to be upregulated during light-induced retinal degeneration and to be reduced after neuroprotective treatments. Thus, caspase-1 may be proapoptotic in the retina. To test directly the role of caspase-1 in photoreceptor apoptosis, three mouse models were analyzed for retinal degeneration in the presence or absence of caspase-1.

**METHODS.** Photoreceptor apoptosis was monitored in one model of induced (exposure to light) and in two models of inherited (rd1, VPP) retinal degeneration. Retinal degeneration was assessed qualitatively by light microscopy and quantitatively by the determination of free nucleosomes with ELISA or by rhodopsin measurements. Gene expression and protein levels were assessed by real-time RT-PCR and by Western blot analysis, respectively.

**RESULTS.** Levels of caspase-1 proenzyme increased in all models of retinal degeneration concomitantly with the onset of cell death. Maturation or classic activity of caspase-1 was not detected in the retina. Ablation of caspase-1 was protective in the model of adRP (VPP mouse), but not in the two other models. Ablation of interleukin-1 receptor type 1 was without effect. Expression of monocyte chemoattractant protein (MCP)-1 increased in the model protected by caspase-1 ablation.

**CONCLUSIONS.** Increased retinal expression of caspase-1 proenzyme may be a common marker for photoreceptor degeneration. The differential effects of caspase-1 ablation suggests a modulatory role of caspase-1 for photoreceptor apoptosis in some but not all models. Such a modulatory activity may involve a caspase-1 function different from the classic activation of interleukin-1β. (Invest Ophthalmol Vis Sci. 2006;47: 5181–5190) DOI:10.1167/iovs.06-0556

Photoreceptor cell death is a hallmark of most retinal dystrophies. Dying photoreceptors share all the major morphologic features of apoptosis including nuclear condensation and nucleosomal DNA fragmentation, cytosolic condensation, and cell shrinkage as well as the formation of apoptotic bodies. Apoptosis has been considered as the final common pathway of cell death in retinal degenerations. The intracellular signals that lead to apoptosis of the visual cells are still far from conclusive. The caspases, a family of cysteine proteases, have been implicated in the regulation of apoptotic cell death in a variety of systems. Caspase-dependent cell death is regulated via one of three major pathways: the receptor- or the mitochondria-mediated cell signaling pathway or by an endoplasmic reticulum stress response. One of the key events triggering caspase-dependent apoptosis is the activation of initiator caspases by oligomerization of the inactive cellular precursors in response to death stimuli. Once activated, initiator caspases induce a tightly regulated cascade of events propagating the death signal by activation of downstream effector caspases leading to cleavage of various cellular substrates in a process that culminates in cell death (reviewed in Ref. 4).

In experimental models developed to study cell death in the retina, several different caspases—in particular, caspase-3—have been reported to be activated during apoptosis. These models include cell death induced by ischemia, excitotoxicity, treatment with autoantibodies, and lead and calcium overload. Caspase activation has also been demonstrated in some animal models of inherited retinal degeneration such as rhodopsin S334ter rats, Royal College of Surgeon (RCS) rats, tubby mice, and rd6 mice. For retinal degeneration in the rd1 mouse, which carries a mutation in the gene encoding the phosphodiesterase β-subunit (PDE6β), contradictory data regarding the involvement of caspase-3 have been reported. Both caspase-dependent and independent photoreceptor cell death have been described.

Caspase (Casp)-1 has been identified as the mammalian homologue to the nematode ced-3 gene and has been implicated in programmed cell death. Today, Casp-1 is mainly associated with inflammation, for its ability to cleave the inactive 31-kDa cytokine pro-interleukin-1β (pro-IL-1β) into an active 17-kDa mature form. However, evidence of an involvement of Casp-1 also in apoptosis has been demonstrated in several in vitro and in vivo systems. Using the model of light-induced retinal degeneration, we and others have shown that Casp-1 gene expression is increased in retinas injured by exposure to light and that treatments that increase the resistance against light-induced damage (e.g., hypoxic preconditioning) prevent increased Casp-1 expression. The findings suggest that Casp-1 is involved in the mediation or regulation of photoreceptor apoptosis after a light-induced insult.

In the present study, we tested whether Casp-1 was induced in other models of retinal degeneration as well and whether ablation of Casp-1 would influence the degenerative process. For this purpose, we used the rd1 mouse as a model for autosomal recessive retinitis pigmentosa (arRP) and the VPP mouse, representing a model for autosomal dominant RP (adRP). The rd1 mouse develops a fast and light-independent degeneration of photoreceptors starting around postnatal day (PND)10.15 The VPP mouse expresses a mutant rhodopsin transgene encoding a protein with three amino acid substitutions (V20G, P23H, and P27L) one of which (P23H) repre-
sents the most common cause for adRP in the United States. The expression of the transgene leads to photoreceptor death with loss of function.30-31 Retinal degeneration in the VPP mouse is accelerated by light45-55 but proceeds slower than in the rd1 mouse. We analyzed the course of retinal degeneration in the light-induced and the two inherited models in the presence and absence of functional Casp-1 and showed that the lack of Casp-1 activity affected the retina differentially in the three models.

**MATERIALS AND METHODS**

**Animals**

All procedures concerning animals were in accordance with the regulations of the Veterinary Authority of Zurich and with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research. All animals were raised in cyclic light (12:12 hours; 60 lux at cage level).

BALB/c mice were purchased from Harlan (Horst, The Netherlands). Casp-1-deficient mice (Casp-1−−) are described elsewhere54 and were generously provided by Winnie Wong (BASF Biosearch, Worcester, MA). VPP mice (generously provided by Muna Naash, University of Oklahoma, Oklahoma City, OK) and wild-type mice (both albino strains) or to 13 klux/2 hours (pigmented, with dilated pupils) were generously provided by Winnie Wong (BASF Bioresearch, Worcester, MA). VPP mice (generously provided by Muna Naash, University of Oklahoma, Oklahoma City, OK) and wild-type mice (both albino strains) or to 13 klux/2 hours (pigmented, with dilated pupils) were generously provided by Winnie Wong (BASF Bioresearch, Worcester, MA) and wild-type mice (both albino strains) or to 13 klux/2 hours (pigmented, with dilated pupils). VPP mice (generously provided by Muna Naash, University of Oklahoma, Oklahoma City, OK) and wild-type mice (both albino strains) or to 13 klux/2 hours (pigmented, with dilated pupils).

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**Light damage was induced in dark-adapted 6- to 8-week-old mice placed in cages with reflective interior by exposure to 5 klux/1 hour (albino strains) or to 13 klux/2 hours (pigmented, with dilated pupils) of diffuse white fluorescent light, as described elsewhere.57 The extent of light-induced damage in the retinas after different recovery periods in darkness was quantified by the ELISA-based determination of free nucleosomes in the cytoplasm (generated after internucleosomal cleavage of genomic DNA during the course of apoptosis), with a cell viability kit (Cell Death Detection Kit no. 1920685; Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s recommendation or by the determination of the rhodopsin content after 16 hours of dark adaptation as described.58**

**Qualitative assessment of retinal tissue was performed by light microscopic viewing of sections from the lower temporal retina of 2.5% glutaraldehyde-fixed and Epon 812-embedded eyes, as has been described.59**

**Retinas were homogenized in 100 mM Tris/HCl (pH 8.0) and analyzed for protein content by using the Bradford method. Standard SDS-PAGE and Western blot analysis were performed. Polyclonal rabbit anti-casp-1, -3, -7 (kindly provided by Peter Vandenabeele, Ghent University, Belgium), anti-casp-1 (p10) (sc-514; Santa Cruz Biotechnology, Santa Cruz, CA), anti-casp-9 (no. 9504; Cell Signaling, Danvers, MA) and sheep anti-IL1β (S329/B4b, kind gift from Stephen Poole; NIBSC [Institute for Biological Standards and Control], Potters Bar, UK) antibodies were applied, followed by a horseradish peroxidase (HRP)-conjugated secondary anti-rabbit (no. NA934; GE Healthcare, Munich, Germany) or anti-sheep/goat (STAR88P; Serotec, Oxford, UK) antibody. To control for equal protein loading, membranes were reprobed with goat anti-actin antibody (sc-1616; Santa Cruz Biotechnology). Immunoreactivity was visualized using a Western blot detection kit [Renaissance; PerkinElmer Life Sciences, Emeryville, CA].**

**Determination of WEHD-pNA Cleavage**

Retinas were dissected and homogenized in 120 μL of cold lysate buffer (BF14100; R&D Systems, Minneapolis, MN) followed by incubation on ice for 30 minutes. The retinal homogenates were centrifuged at 15,000g for 10 minutes at 4°C. The protein content of supernatants was determined by a protein assay (Bio-Rad, Hercules, CA) using bovine serum albumin as the standard. An equal quantity (150 μg) of retinal protein was loaded into each well of a microtiter plate. Lysates were incubated with an equal volume of 1× reaction buffer and Casp-1

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| Table 1. Primer Sequences |
FIGURE 1. Retinal apoptosis and expression of caspases after exposure to light. Dark-adapted BALB/c (A, C, D) or 129.B6 (B) mice were not exposed (dark) or were exposed to 5 klux of white fluorescent light for 1 hour (A, C, D) or to 13 klux for 2 hours (B). Retinas were harvested immediately (I) or at different time points as indicated. (A) Cell death in the retina was detected by ELISA-based measurement of free nucleosomes in cytoplasmic extracts. Release of nucleosomes was detected as early as 6 hours after exposure and peaked at 36 hours. Data points represent the OD_{405} of retinas of individual mice. Note the logarithmic scale on the y-axis. (B) Retinal morphology of 129.B6 mice at the indicated time points before (dark) or after exposure to light. (C) Apoptotic cell death was associated with accumulation of the Casp-1 proform. Western blots show proforms of individual caspases at different time points after exposure to light as indicated. Levels of Casp-1 increased steadily, whereas expression of other tested caspases remained constant. Representative Western blot analyses from at least three independent experiments are shown. Membranes were reprobed with β-actin to control for equal protein load. (D) Long-term follow-up of Casp-1 expression after exposure to light. Expression of Casp-1 peaked at 5 days after illumination before basal levels were reached after 20 days. β-Actin was tested as loading control.

FIGURE 2. Caspase-1 activity was not detected after exposure to light. Dark-adapted BALB/c mice were not exposed (dark) or were exposed for 1 hour to 5 klux, and retinas were harvested immediately (I) or at different time points thereafter as indicated. (A) Levels of IL-1β mRNA increased after exposure and peaked at 12 hours after illumination. Relative mRNA levels were assessed by exponential RT-PCR (mean ± SD of three reactions performed on a pool of RNAs from three retinas isolated from three different animals per time point). (B) IL-1β was not processed after light-induced damage. Increasing pro-IL-1β levels were detected by Western blot analysis with increasing time (as indicated) after exposure to light (top). Mouse recombinant mature IL-1β (17 kDa) was used as a positive control (pos) for immunoblot analysis. Mature IL-1β was not detected in retinal extracts. *31 kDa proform of IL-1β, **unspecific signal, ***mature form of IL-1β. β-Actin served as the loading control (bottom). (C) Casp-1 was not enzymatically active after light-induced damage. WEHD-pNA cleavage was determined spectrophotometrically in vitro using an ELISA-based method on retinal extracts prepared from control (dark) or from retinas isolated at different time points after exposure to light, as indicated. pos: recombinant active Casp-1 as the positive control (the mean ± SD of three retinas from three different animals). (D) Pro-Casp-1 was not processed after exposure to light. Retinal extracts prepared at 72 hours after exposure to light were tested with two antibodies recognizing the Casp-1 proform and p20 active form (left) or the Casp-1 proform and the p10 active form (right). Extract of Casp-1 knockout mice (KO) was loaded for specificity control, and β-actin served as the loading control (bottom). pos: recombinant active Casp-1 as positive control for antibodies.
substrate, according to the manufacturer’s instructions at 37°C for 18 hours. Cleavage of the peptide substrate WEHD-pNA was monitored by liberation of the chromogenic pNA measuring absorption at 405 nm. Three animals were analyzed per condition.

RESULTS

To establish the time course of light-induced photoreceptor apoptosis, we used two different methods. To quantify the amount of cells, which irreversibly entered the process of apoptotic cell death, that is cells in the phase of internucleosomal DNA cleavage, we measured the release of free nucleosomes in retinal lysates by ELISA. To assess cell loss and morphologic changes by apoptosis in the retina, we used classic histologic methods.

Nucleosomal release above background was detectable at 6 hours after illumination and peaked at 36 hours after exposure to light (Fig. 1A) as described earlier. Signal intensity declined slowly thereafter, but relatively high levels remained up to 3 days after illumination (the last time point tested). Morphologic changes in light-exposed retinas were detectable by light microscopy at 1 day after exposure (Fig. 1B). Rod outer segments (ROS) and rod inner segments (RIS) were severely disturbed, and many photoreceptor nuclei in the outer nuclear layer (ONL) showed condensed chromatin. At 2 days after illumination, the ONL was thinned and contained predominantly nuclei with condensed chromatin. The area of ROS and RIS showed complete devastation. The gap between ONL and the pigment epithelium (PE) at days 2 and 5 may be preparation artifacts resulting from the weakened retinal structures. Between 2 and 5 days after exposure, most photoreceptor nuclei were lost. Between 5 and 10 days, debris of dead cells was removed, and no further change was obvious up to day 30 after the exposure.

DNA cleavage, as detected by the cell death detection ELISA assay, precedes the removal of the affected cell. Its peak was detected between 1 and 3 days after the insult, whereas physical loss of cells followed after a delay of approximately 1 day (peak between 2 and 5 days). Accordingly, we focused on the first 3 days after exposure to light for the analysis of biochemical events in the signaling cascades leading to cell death.

Expression and Activity of Caspase-1 in Retinal Degeneration

In many systems, apoptotic cell death is mediated by caspases. In the light-induced damage system, however, Western blot analysis revealed that the amount of the proenzymatic forms of caspase-3, -6, -7, and -9 remained unchanged over a period of 3 days after the exposure to light (Fig. 1C). In addition, cleaved (activated) fragments of these caspases remained undetectable. Together with results from others, this suggests that light-induced damage is independent of these caspases. In contrast, increasing Casp-1 proenzyme levels were detected after light treatment (Fig. 1C). Induction of Casp-1 followed the initial time course of nucleosomal release (Fig. 1A) starting at 6 hours after illumination. In contrast to the release of nucleosomes, Casp-1 proenzyme increased continuously, reaching a maximum at 5 days. Thereafter, pro-Casp-1 levels declined to baseline (Figs. 1C, 1D). Thus, elevated Casp-1 levels were present beyond the peak of apoptosis.

The major known role of active Casp-1 is proteolytic processing of IL-1β from a 33-kDa precursor protein to the 17-kDa mature form. Light exposure induced expression of IL-1β, with peak mRNA levels at 12 hours (Fig. 2A) and maximum protein (proform) levels at 24 to 72 hours after illumination (Fig. 2B). Although both Casp-1 protease and IL-1β substrate were induced and present in high amounts at 72 hours after illumination, we did not observe mature IL-1β by Western blot analysis (Fig. 2B) or ELISA (not shown). Using an in vitro assay for WEHD-pNA substrate cleavage, we also did not detect increased Casp-1 activity in retinal extracts after exposure to light (Fig. 2C). Because Casp-1 has to be cleaved to become active, we tested for the presence of the mature form of Casp-1 in retinal extracts prepared 72 hours after illumination. Using two different antibodies that recognized cleaved Casp-1 products in control experiments (p20 and p10, respectively), we did not detect any cleaved products of Casp-1 in retinal extracts (Fig. 2D). The lack of IL-1β substrate cleavage and of the cleved Casp-1 mature form suggests that Casp-1 did not mature and was therefore not activated, despite the increased expression of the proform.

To test whether increased Casp-1 levels is be a general phenomenon of retinal degeneration, we also analyzed its expression in two models of inherited retinal degeneration.
The rd1 mouse is characterized by fast photoreceptor degeneration, beginning at approximately postnatal day 10 (PND10) and peaking at ~PND13, whereas retinal degeneration in the transgenic VPP mouse has a later onset, and degeneration proceeds more slowly (compare the images in Figs. 5A and 5B). Although retinal degeneration in the rd1 mouse may or may not involve Casp-3, the molecular pathways of photoreceptor apoptosis in the VPP mouse have not been investigated so far. Whereas wild-type animals expressed low amounts of Casp-1 protein that remained unchanged at all time-points, increased levels of Casp-1 were found early after onset of degeneration in rd1 and VPP retinas (Figs. 3A, 3B). In both models of inherited retinal degeneration, Casp-1 levels increased further with age. Of note, Casp-1 levels in the rd1 retina where highest at PND37, at a time point when loss of photoreceptors was nearly complete (see Fig. 5A). This suggests that Casp-1 was expressed in cells different from photoreceptors.

Similar to the light-damage model, we did not observe cleaved mature Casp-1 (not shown). Furthermore, in vitro cleavage assays using retinal extracts from wild-type and VPP mice at PND28 again pointed to the absence of classic Casp-1 activity (Fig 3C).

In each of the three models of retinal degeneration, increased levels of Casp-1 coincided with the onset of photoreceptor apoptosis. Levels of Casp-1 remained high, at least until the degenerative process was completed.

**Role of Casp-1 in Retinal Degeneration**

Previously, we have shown that hypoxic preconditioning suppresses transcriptional upregulation of Casp-1 after exposure to light and protects photoreceptors from light-induced damage. These findings suggest that pretreatment may protect retinal morphology and function by interfering with a Casp-1-dependent pathway. To test the suggested role of Casp-1 in light-induced photoreceptor apoptosis, we exposed Casp1–/– and wild-type control mice, both on a mixed 129/Sv-B6 genetic background, to high levels of white light (2 hours, 13 klux) and analyzed their retinal morphology at 5 and 10 days after exposure. Absence of Casp-1 did not notably influence light-induced retinal degeneration, as progression and extent of degeneration was comparable to wild-type mice (Figs. 4A, 4B). Retinas of both genotypes, analyzed 5 days after exposure to light, were characterized by a thinned ONL and a complete disintegration of RIS and ROS. Most of the remaining photoreceptor nuclei were pyknotic, indicating ongoing cell death. Photoreceptor cells were mostly cleared from the retina 10 days after illumination. During all times, the inner retinal structures were well preserved.

As the biochemical assays used to detect mature Casp-1 and IL-1β (Figs. 1, 2) may not have been sensitive enough to detect small amounts of active proteins, we addressed a potential role of IL-1β in retinal degeneration by using IL-1 receptor type 1 knockout mice (IL-1R1–/–) on a BALB/c genetic background. BALB/c wild-type mice served as the control. As IL-1R1 is the only identified signaling receptor mediating the effects of IL-1β, its absence should completely prevent any IL-1β signaling.
Casp1–/– and VPP/Casp1 –/– double-mutant mice and investi-
gated retinal morphology at different postnatal days. Retinas of
VPP mice exceeded similarly in rd1/Casp1–/– retinas (Table 2) and retinal morphology (not shown). This finding
supports the conclusion that Casp-1 may influence retinal de-

generation in the VPP mouse independent from its classic function as activator of IL-1β signaling.

Signaling Mechanisms

To address potential signaling mechanisms involved in such a nonconventional Casp-1 function, we tested expression of sev-
eral genes involved in cytokine or chemokine signaling by real-time PCR. In retinas of wild-type animals, Casp-1 gene
expression remained stable and at low levels during postnatal development and aging (Fig. 6). In VPP mice, however, in-
creased Casp-1 gene expression was detected from PND21 (4-fold) to PND56 (10-fold), which is in line with the increased
levels of Casp-1 protein detected by Western blot analysis (Fig. 3B). The oligonucleotides used for real-time PCR hybrizide
upstream of the neomycin insertion (exon 6,34) in the Casp-1 gene-coding region, but because of the lack of a negative feedback
inhibition that may be present in the VPP mouse retina as judged by the amount of rhodopsin at PND21 and PND24
(Tab. 2) and retinal morphology (not shown). This finding
supports the conclusion that Casp-1 may influence retinal de-

genation in the VPP mouse independent from its classic function as activator of IL-1β signaling.

Although the potential existence of alternative signaling mech-
nisms has been discussed,37,48 Wild-type and IL-1R1–/– mice
were exposed to 5 klux of light for 1 hour and analyzed 5 and 10 days after exposure. Retinal degeneration was comparably
severe in both types of mice (Figs. 4C, 4D).

However, Casp-1 did not prevent light-induced photoreceptor degeneration and suggest that IL-1β signaling may not be involved in the degenerative process.

Several molecular pathways exist for cell death in different forms of retinal degeneration.2–41 Therefore, we also tested the potential effect of Casp-1 ablation in the two models of inher-
ited retinal degeneration. For this purpose, we generated rd1/Casp1–/– and VPP/Casp1–/– double-mutant mice and investi-
gated retinal morphology at different postnatal days. Retinas of
Casp1–/– mice, used as controls in this experiment, showed a

normal morphologic appearance (Fig. 5A, top). Ablation of Casp-1 did not affect the course of retinal degeneration in rd1 mice, as the process proceeded without notable differences in
rd1 (Fig. 5A, middle) and rd1/Casp1–/– double-mutant mice (Fig. 5A, bottom). Both rd1 and rd1/Casp1–/– retinas showed scattered apoptotic nuclei at PND11 and almost complete loss of
photoreceptors by PND21. In contrast, ablation of Casp-1 substantially protected retinal morphology in VPP mice (Fig. 5B). At PND21, the ONL thick-
ness in retinas of VPP mice was about half that of Casp1–/– (compare top rows of Figs. 5A and 5B) or of wild-type mice.

Most of the outer segments were severely disturbed. Accord-
ingly, reduced levels of rhodopsin (17% compared with wild-type or Casp1–/–) were detected at this age (Table 2). VPP/Casp1–/– double-mutant mice, however, had more photo-

receptor nuclei, better preserved outer segments and higher levels of rhodopsin (twice as much) than VPP mice. Preservation
of retinal morphology and higher rhodopsin contents persisted until at least PND42 (Fig. 5B, Table 2). At PND56, the
ONL was still thicker in double-mutant mice, but the structure of the outer segments was severely disturbed, which was
reflected by a rhodopsin content similar to that of VPP mice. Although lack of Casp-1 protected the retina of VPP mice, morphology and rhodopsin content did not reach the quality
or the amount, respectively, of wild-type or Casp1–/– animals. The continuous expression of mutant rhodopsin obviously prevented a complete protection.

Because lack of Casp-1 protected the VPP retina even though we did not observe Casp-1 cleavage activity in VPP mice in in vitro assays (Fig. 3C), we also analyzed VPP/IL-1R1–/–
double mutant animals to address a potential role IL-1β signal-
ing for VPP mediated retinal degeneration. Lack of IL-1R1 did not influence the degeneration in the VPP mouse retina as judged by the amount of rhodopsin at PND21 and PND42 (Table 2) and retinal morphology (not shown). This finding
supports the conclusion that Casp-1 may influence retinal de-

generation in the VPP mouse independent from its classic function as activator of IL-1β signaling.
involved in the activation of pro-Casp-1.59 Recently, asc has also been implicated in the activation of Casp-8.50 In contrast to the decreasing expression in wild-type mice, VPP mice show a two-fold increase of asc expression with time (Fig. 6). In mice lacking functional Casp-1, asc expression is almost undetectable (Fig. 6) suggesting that Casp-1 protein is necessary to stimulate asc gene expression. The increase of asc in VPP mice also further supports an involvement of Casp-1 in the degenerative process.

Ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF)-2 have been implicated in retinal neuroprotection (reviewed in Ref. 41). Expression levels of CNTF were similar in all four strains tested (Fig. 6). Only at PND56, both VPP and

Table 2. Rhodopsin Content in Retinas of Dark-Adapted Mice with Different Genotypes

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<tr>
<td>VPP/IL-1R−/−</td>
<td>0.08 ± 0.02 nmol</td>
<td>ND</td>
<td>0.05 ± 0.03 nmol</td>
<td>ND</td>
</tr>
<tr>
<td>19%</td>
<td>ND</td>
<td>10%</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4/4</td>
<td>12/8</td>
<td>10/6</td>
<td>9/6</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data for each retina are given as the mean ± SD. Data for wild-type mice were set to 100%, and rhodopsin levels of the different genotypes were calculated as percentages of wild-type levels at a particular age. Numbers divided by virgules represent the number of retinas analyzed and the number of different animals used, respectively. Differences in rhodopsin content were significant between VPP and VPP/Casp1−/− mice at PND21, -28, and -42 (unpaired t-test with Welch correction, P ≤ 0.05). ND, not determined.

Figure 6. Gene expression in wild-type and single- and double-mutant mice. Relative levels of indicated RNAs in retinas of wild-type, Casp1−/−, VPP, and VPP/Casp1−/− at PNDs 11, 15, 21, 28, 42, and 56, as determined by real-time RT-PCR. mRNA levels are expressed relative to those of the 11-day-old wild-type samples, which was set to 1. RNAs isolated from three independent retinas per time point and genotype were pooled, and amplifications were performed in triplicate (means ± SD).
VPP/Casp1−/− showed a tendency of increased CNTF expression. Because expression increased similarly in both strains, especially at the earlier time points, CNTF may not be a factor responsible for the observed protection by ablation of Casp-1. In contrast to CNTF, expression of FGF-2 responded strongly to the presence of the VPP transgene: Expression was induced 30- to 50-fold beginning at PND21, concomitantly with the onset of the degeneration (Fig. 6). At PND21 and PND28, expression in double-mutant mice was slightly reduced compared with that in VPP mice. This finding may reflect the reduced severity of retinal injury at this time point in these mice.

Monocyte chemotactic protein (MCP)-1 has been implicated in the recruitment of macrophages to the site of injury. Because the lack of MCP-1 has been reported to cause an AMD-like phenotype in aged mice, MCP-1 may be needed to maintain normal retinal morphology. Whereas expression of MCP-1 was at basal levels in wild-type and Casp1−/− animals at all time points tested, its expression strongly increased in VPP (from PND21) and especially in VPP/Casp1−/− mice (from PND15; Fig. 6). At all time-points tested (except PND11), expression of MCP-1 was stronger in the VPP/Casp1−/− animals. Such an increased expression was not detected in Casp−1−/− animals but only occurred in combination with the disease-causing transgene. Therefore, lack of Casp-1 did not result in an increased MCP-1 expression per se, suggesting that MCP-1 could be one factor, which may play a role in the protective effect of Casp-1 ablation in VPP mice.

**DISCUSSION**

We show that Casp-1 RNA and protein are upregulated in three different models of retinal degeneration (light-induced, rd1, VPP). Our data suggest that an injured retina increases expression of the proform of Casp-1 and that this proform may exhibit a nonclassic activity different from IL-1β cleavage. Ablation of Casp-1 slowed the disease progression only in the model with the mildest form of retinal degeneration (VPP). However, the protection observed in VPP/Casp−/− animals was only transient suggesting that additional pathways, independent of Casp-1, are involved in the regulation of retinal degeneration.

Lack of Casp-1 did not protect photoreceptors after acute light damage, in which cell death is synchronous and proceeds quickly and after which most photoreceptors are cleared from the subretinal space within 10 days. Also in the rd1 model, loss and clearance of photoreceptors occurs roughly in the same time frame, and photoreceptors are not protected by the ablation of Casp-1. In contrast, apoptosis of visual cells in the VPP mouse proceeds slowly over an extended period. In addition and contrary to light damage, cell death in the VPP mouse depends on phototransduction but not on c-Fos. Thus, significant differences in the signaling of apoptosis exist between these two models, and it appears that Casp-1 rather affects death pathways causing retinal degeneration of a milder phenotype with a slow progression. This is in line with results showing that lack of Casp-1 renders neonatal mice resistant to moderate but not to severe cerebral hypoxia-ischemia insults.

Even though degeneration of the ONL may proceed at different speeds in the various models, the death process may nevertheless be similar on the single cell level once apoptosis is induced. Therefore, the amount of dying cells at any given time may be critical for the protective effect observed by the ablation of Casp-1. Recent theoretical and mathematical evaluations of neuronal cell death in several models of inherited retinal degeneration and other neurologic disorders suggested that a mutation imposes a mutant steady state and that a single event randomly induces cell death. This model has been refined by the inclusion of diffusible death factors to explain patchy cell death as it often is observed in patients with RP and in animal models. Such diffusible factors or at least cell-cell interactions between dying and neighboring cells have been postulated earlier and bystander effects were suggested to play an important role in neuronal cell death in the retina as well. Similarly, the magnitude of the cell death stimulus is a determining factor deciding over an acute or a chronic fate. Based on these reflections, we propose that lack of Casp-1 may influence a bystander effect and/or may play a role in the production, release or activity of potential death factors. The nature of the cells that may be involved in such an effect remains to be determined, but the high expression of Casp-1 in rd1 retinas at PND27 (Fig. 3A) when most photoreceptors have been cleared from the retina (Fig. 5A) suggests strong Casp-1 expression in cells different from photoreceptors. In contrast, recent reports rather convincingly demonstrate high expression of Casp-1 in the ONL in several models of retinal injury. Clearly, a careful and more elaborate study of retinal Casp-1 expression is needed to unravel the complete role of Casp-1 in disease induction and progression.

In models with almost synchronous cell death as in the light-damage model and the rd1 mouse, such effects and/or factors may be of such concentrations that lack of Casp-1 may not be able to influence their activity sufficiently. In models of slow photoreceptor degeneration, however, the reduced amount of dying cells per time may allow protection by the lack of Casp-1.

The initial analysis of factors that could be involved in the protection observed in the VPP/Casp1−/− double mutant mice revealed a striking difference in the expression of MCP-1. Expression of this chemokine is induced in the degenerating VPP retina, and lack of Casp-1 seems to stimulate its mRNA production even further (Fig. 5D). Whether MCP-1 may be involved in the protection of photoreceptors in VPP/Casp1−/− double-mutant mice remains to be shown, but it is interesting to note that lack of MCP-1 has been reported to lead to an AMD-like phenotype, including photoreceptor atrophy. Even though such a phenotype manifests itself only in aged animals, MCP-1 may nevertheless play a role in the survival of retinal cells, as has been suggested for central nervous system neurons, and/or MCP-1 may be involved in maintaining the integrity of the retinal structure.

VPP mice express a human transgene encoding a mutant rhodopsin with three amino acid substitutions (V20G, P25H, P27L), one of which (P22H) is the most prevalent mutation in patients with RP. The proposed mechanism of P22H-mediated degeneration involves formation of protein aggregations and proteasome-processing defects. This mechanism links this form of RP to a broad class of neurodegenerative diseases characterized by accumulation of misfolded protein aggregates such as Alzheimer disease, Parkinson disease, Huntington disease, prion encephalopathies, and amyotrophic lateral sclerosis (ALS). Casp-1–mediated pathways and increased expression of MCP-1 have been suggested to play a role in a variety of these acute and chronic neurologic disorders, similar to the results presented herein.

It has been generally accepted that the common event for caspase activation is proteolysis of the proenzyme at internal aspartate residues producing, in the case of Casp-1, mature p20 and p10 forms. However, four lines of evidence suggests that in the retina, Casp-1 may not exhibit classic activity: First, p10 and p20 cleavage products were not detected with two different antibodies, as reported earlier by others; second, we did not detect any cleavage activity in retinal extracts; third, we did not observe any mature IL-1β, although all models showed increased IL-1β mRNA levels; and fourth, lack of IL-1R1 did not influence retinal degeneration, suggesting that mature IL-1β,
the product of classic Casp-1 activity, is not involved in initiation and/or progression of the degeneration. Instead of mature Casp-1, we detected overaccumulation of the Casp-1 proform. Based on these data and the neuroprotective effect of Casp-1 ablation in the VPP mouse, we speculate that Casp-1 may orchestrate mechanisms of cell death independent of IL-1β maturation. It has been reported for example that the CARD domain of the procenzyme interacts with RIP2 and activates NFκB independently of enzymatic activity. 67, 68 Other mechanisms are possible as well.

Recent work has revealed that initiator caspases may be activated through oligomerization and a conformational change independent of a cleavage activity. 69, 70 Similarly, pro-Casp-1 can be activated through oligomerization in vitro 71 a process facilitated in vivo by asc. 72 Overexpression of asc can lead to apoptotic cell death, and asc is silenced in human breast carcinoma and glioblastoma cells. 73–76. In the retina of VPP mice, asc is expressed at higher levels compared with controls, whereas asc expression is almost completely silenced in mice lacking functional Casp-1 (Fig. 6).

In summary, we showed that increased expression of Casp-1 may be a common marker for retinal degeneration, and that ablation of Casp-1 is neuroprotective, at least in a model of a comparably mild form of photoreceptor degeneration. It is important to establish the generality of these observations and to test whether interference with classic or nonclassic Casp-1 function may be an option to protect retinal cells in slowly progressing retinal degenerations.

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