Effect of Aqueous Humor on Apoptosis of Inflammatory Cell Types

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PURPOSE. To determine whether aqueous humor promotes cell death in cells involved in inflammatory responses.

METHODS. Multiple immune cell types, most characteristically involved in inflammatory responses, were incubated for 24, 48, and 72 hours in the presence or absence of 50% aqueous humor. Promotion of cell death was assayed by staining for an early indicator of apoptosis. The percentage of cells undergoing apoptosis was measured by flow cytometry. To identify partially the apoptosis-inducing factor, aqueous humor was pretreated with proteinase K to degrade protein. In other experiments, aqueous humor was fractionated by centrifugation on filters capable of separating molecules above and below 10 kDa or 30 kDa kilodaltons in size.

RESULTS. Rabbit aqueous humor promoted apoptosis in a wide variety of immune cells, including lymphokine-activated natural killer cells, resting T cells, an activated T-cell line, RAW 264.7 and J774A0.1 monocyte-macrophage cell lines, and neutrophils. As previously shown, aqueous humor did not promote apoptosis of murine corneal endothelial cells. Apoptosis was also not induced in human corneal endothelium, mouse corneal epithelium, or iris/ciliary body cell lines. Instead, aqueous humor partially protected these ocular tissues from starvation-induced cell death. Pretreatment with proteinase K inhibited the apoptosis-inducing activity. Moreover, the apoptosis-inducing activity segregated with the aqueous humor fraction containing molecules less than 10 kDa in size.

CONCLUSIONS. These data show that aqueous humor contains a factor or factors that promote death of cells that participate in inflammatory processes. By contrast, ocular tissues, such as the corneal endothelium and iris/ciliary body, are impervious to aqueous humor-induced cell death. The aqueous humor-borne factor(s) may contribute to the immune privilege of the anterior chamber by purging potential inflammatory cells. (Invest Ophthalmol Vis Sci. 1999;40:1418-1426)
The demonstration of an antiapoptotic factor in AH presents a teleological quandary to immune privilege in the AC. As previously described, apoptosis of inflammatory cells is imperative for the maintenance of ocular immune privilege.13-16 The prevention of apoptosis in nonmitotic ocular tissues, however, is crucial for the preservation of sight. This dilemma led to the hypothesis that inflammatory cells invading the eye, such as neutrophils, macrophages and T cells, and natural killer (NK) cells are not protected by the antiapoptotic factor(s) present in AH. Rather, it was postulated that AH contains one or more factors that promote cell death in nonocular immune cells. A corollary of this hypothesis predicts that ocular tissues, such as iris/ciliary body cells, are immune to the putative proapoptotic effect of AH. In this manner the AH would contribute to the prevention of intraocular inflammation and the overall maintenance of immune privilege while satisfying the need to preserve the integrity of ocular tissues.

**METHODS**

**Animals**

Ten- to 12-week-old Balb/c mice were obtained from the mouse colony at the University of Texas Southwestern Medical Center, Dallas. Severe combined immune deficiency (SCID) mice on a C57BL6 background were generously provided by Michael Bennett, Department of Pathology, University of Texas Southwestern Medical Center. New Zealand White rabbits were kindly provided by James V. Jester, Department of Ophthalmology, University of Texas Southwestern Medical Center. All animals were treated in accordance with the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Aqueous Humor**

Aqueous humor was extracted from freshly enucleated and washed (1% iodine solution) rabbit eyes (Pel Freeze Biologics Division, Fayetteville, AR) with a 27-gauge needle and syringe. Fresh AH was obtained from the anterior chamber of anesthetized New Zealand White rabbits by paracentesis using a 27-gauge needle and syringe. All AH was stored in siliconized vials at −80°C before use.

**Viability Assay**

Cells were incubated for 24, 48, and/or 72 hours in 6- or 12-well tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ) in medium containing 50% AH or 50% Hank's balanced salt solution (HBSS) as a starvation control. In some experiments, the AH was pretreated with 10 μg/ml proteinase K (Oncor, Gaithersburg, MD) for 1 hour at 37°C to inactivate all protein. Proteinase K was inactivated by the addition of medium containing 10% fetal bovine serum (HyClone, Logan, UT). In other experiments, AH was size fractionated by centrifugation on microconcentrators (Microsep; FiltroN, Northborough, MA) with filter membrane cutoff sizes of 10 kDa or 30 kDa. Briefly, 10 ml AH was applied to the top chamber and centrifuged at 3000g for 60 minutes. Both top and bottom fractions were reconstituted to the original sample volume in 0.1% bovine serum albumin before testing for proapoptotic activity.

**Cell Lines**

Two monocyte-macrophage cell lines originally derived in Balb/c mice, RAW 264.7 (TIB 71) and J774A.1 (TIB 67), were purchased from American Type Culture Collection (ATCC), Rockville, MD. The Jurkat clone E6-1 (TIB 152, ATCC) was originally derived from the Jurkat-FHCRC cell line of human acute T-cell leukemia. These cells are capable of producing large quantities of interleukin (IL)-2 and interferon (IFN)-γ and are therefore used as a model of activated T cells.39 Jurkat, RAW 264.7, and J774A.1 cells were maintained in complete RPMI-1640 (JRH Biosciences, Lenexa, KS) containing 10% heat-inactivated fetal bovine serum (HyClone), 2 mM L-glutamine, 10 mM HEPES buffer solution, 1 mM sodium pyruvate solution (all from JRH Biosciences), 1% nonessential amino acids solution, and 1% penicillin-streptomycin-fungizone solution (both from BioWhittaker, Walkersville, MD). Human and mouse corneal endothelial cell lines and mouse epithelial cell lines were previously generated by transformation with human papilloma virus E6/E7 genes.30,31 These cells were maintained in complete minimum essential medium (MEM; JRH Biosciences) containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 2 mM MEM vitamins (JRH Biosciences), and 1% penicillin-streptomycin-fungizone solution. Balb/c iris/ciliary body (I/CB) cells were prepared as previously described and were maintained in complete RPMI-1640.32 The I/CB cells were not transformed with human papilloma virus E6/E7 genes.

**T Cells**

Resting T cells were purified from naive Balb/c mice. Briefly, spleen cells were collected and erythrocytes lysed. Whole spleen cell suspensions were incubated on scrubbed nylon wool (Fenwal Laboratories, Deerfield, IL) at 37°C in 5% CO₂ for 1 hour. T cells were eluted with 30 ml HBSS.

**Lymphokine-Activated Killer Cells**

Lymphokine-activated killer (LAK) cells were generated from Balb/c and C57BL/6 SCID mice. Briefly, spleen cells were collected and erythrocytes lysed. Splenocytes were resuspended in complete Dulbecco’s modified Eagle’s medium (JRH Biosciences) containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 2 mM MEM vitamins, and 1% penicillin-streptomycin-fungizone solution and supplemented with 5 × 10^-5 M 2-mercaptoethanol (NCI, Biological Response Modifiers Program, Frederick, MD) and 1000 U/ml recombinant human IL-2 (Hoffman-La Roche, Nutley, NJ). Cells were incubated at 37°C in 10% CO₂ for 4 days. This procedure enriches for NK cells.33 LAK cells generated from T- and B-cell-deficient SCID mice contained more than 80% NK1.1" NK cells, determined by conventional flow cytometry. After 4 days, LAK cells were collected, washed, layered over Histopaque-1083 (Sigma, St. Louis, MO) and centrifuged at 3000 rpm for 30 minutes. The buffy coat was collected and used in subsequent assays.

**Neutrophils**

Neutrophils were collected by peritoneal lavage of normal mice that were injected intraperitoneally with 2.5 ml 3% thioglycollate (Sigma) medium 5 hours earlier. Cells were layered
over 3 ml Histopaque-1083 and spun at 3000 rpm for 30 minutes. Neutrophils were collected in theuffy coat.

**Detection of Apoptosis**

The annexin V assay specifically detects cells in the early stages of apoptosis. This assay is based on the principle that apoptosis is accompanied by a change in the plasma membrane structure resulting in the surface exposure of phosphatidylserine (PS) while membrane integrity remains intact. Phosphatidylserine can be detected and quantified by its affinity to bind annexin V, a phospholipid-binding protein. Phosphatidylserine is also exposed in dead and necrotic cells. Therefore, we used a membrane-impermeable DNA stain, propidium iodide (PI), to identify dead and necrotic cells. Thus, dead and necrotic cells stain with both annexin V and PI, whereas apoptotic cells stain with annexin V. This double-staining method was used to discriminate between vital, apoptotic cells and dead cells. Accordingly, cells were washed with cold PBS and resuspended in binding buffer (R&D Systems, Minneapolis, MN) at 1 x 10⁶ cells/ml. Cells (100 μl) were then incubated with fluorescein-labeled annexin V protein (0.1 μg) and PI (0.5 μg; both from R&D Systems) for 15 minutes at room temperature in the dark. Finally, 800 μl binding buffer was added to each tube. Staining intensity was analyzed on a flow cytometer (FACScan; Becton Dickinson, Mountain View, CA) using CellQuest software (Becton Dickinson) for acquisition and analysis (5000 cells per sample). All results are expressed as the percentage of viable cells undergoing apoptosis ± SEM.

**Statistics**

In all experiments, n ranged from 3 to 4 per group. Differences among groups were analyzed by Student’s t-test. P < 0.05 was considered significant.

**RESULTS**

**Aqueous Humor Induces Apoptosis of Multiple Immune Cell Types**

It was hypothesized that AH contains a factor that guards against inflammation by inducing immigrant immune cells to die. This hypothesis was tested on a variety of immune cell types using an assay that detects an early marker of apoptosis. When cells apoptose, the membrane phospholipid PS is translocated from the inner leaflet of the membrane, where it usually resides, to the outer leaflet. The annexin V protein binds to PS with high affinity and can therefore be used to detect PS+ cells in the early stages of apoptosis. In each of the following experiments, cells were collected and incubated in medium containing 50% AH collected from freshly enucleated rabbit eyes or 50% HBSS to control for dilution. After 24, 48, and 72 hours, cells were collected and stained with fluorescein-labeled annexin V protein to determine the percentage of cells undergoing apoptosis. Figure 1 shows a representative dot plot with annexin V fluorescence on the ordinate (FL1-Height) and PI fluorescence on the abscissa (FL3-Height). Cells were gated into one of four quadrants, each representing a characteristic population. Quadrants A and B represent dead and dying cells (PI positive). Cells in quadrant C are living cells not undergoing apoptosis (annexin V negative and PI negative). Cells in quadrant D (annexin V positive, PI negative) are those in the early stages of apoptosis when PS has flipped to the outer leaflet, but the cell membrane remains intact. The percentage of living cells undergoing apoptosis was calculated by dividing the number of cells in quadrant D by the total number of living cells (quadrants C + D).

We began these studies by testing the effect of AH on the primary constituents of an inflammatory response: T cells, neutrophils, and macrophages. As shown in Figure 2A there was an approximate twofold increase in the percentage of resting T cells undergoing apoptosis in the presence of 50% AH compared with apoptosis in negative controls. The increase in apoptosis was evident by 24 hours and persisted for at least 72 hours (Fig. 2A). The human Jurkat cell line was used as a model of T cells in the activated state. The presence of 50% AH induced a two- to threefold increase in Jurkat cell death over negative controls (Fig. 2B). The effect of AH on Jurkat cells was not seen at 24 hours but became evident by 48 and 72 hours (Fig. 2B). We next tested the effect of AH on the innate mediators of inflammation, neutrophils, and macrophages. The data show a significant increase in the percentage of apoptotic neutrophils incubated in the presence of 50% AH relative to negative controls incubated with 50% HBSS (Fig. 3). The effect of AH was evident beginning at 24 hours and persisted for at least 72 hours (Fig. 3). Two Balb/c monocyte-macrophage cell lines, J774A.1 and RAW 264.7, were used in this study. By 72 hours, more than two times the percentage of viable J774A.1 cells were induced to undergo apoptosis in the presence of 50% AH relative to negative controls (Fig. 4A). A similar result was seen using the RAW 264.7 cell line (Fig. 4B). Together these data show that AH contains at least one factor that triggers apoptosis in multiple cellular mediators of inflammation.
Figure 2. Aqueous humor induces apoptosis of T cells. (A) Resting T cells were purified from the spleens of naive Balb/c mice. T cells were incubated for 24, 48, and 72 hours in medium containing 50% AH or 50% HBSS as a dilution control. At each time point, cells were collected and stained for apoptotic cells by FACS. (B) The Jurkat activated T-cell line was treated and tested as described in part (A). All results are expressed as the percentage of viable cells undergoing apoptosis ± SEM. *P < 0.05 for AH versus HBSS control.

To determine whether the apoptosis-inducing factor affects other immune cell types, we tested the effect of AH on NK cells. Certain tissues surrounding the AC—namely, the corneal endothelium and lens epithelium—are potentially vulnerable to NK-mediated lysis because of their lack of class I major histocompatibility complex molecules. Previous data had shown that AH contains factors, such as transforming growth factor-β and migratory inhibitory factor, which inhibit NK cell function. It was hypothesized that the apoptosis-inducing factor present in AH aids in the preservation of delicate ocular tissues by killing NK cells. Natural killer cell-enriched LAK cells were generated by incubating Balb/c splenocytes in the presence of 2-mercaptoethanol and IL-2 for 4 days. LAK cells incubated in the presence of 50% AH were induced to apoptosis at 24, 48, and 72 hours (Fig. 5A). LAK cells were also generated from C57BL/6 SCID mice to rule out the possibility that T or B cells contaminating the LAK cultures contributed to AH-induced apoptosis. LAK cells generated from SCID mice were also induced to apoptose in the presence of 50% AH (74% versus 45% control apoptosis at 72 hours). These data support the hypothesis that AH offers another layer of protection against NK cell-mediated attack of susceptible ocular tissues surrounding the AC.

To control for the possibility that the proapoptotic factor is released into the AC after death, we tested the effect of fresh AH on LAK cells. Aqueous humor was tapped by paracentesis from anesthetized rabbits. LAK cells were incubated in 50% fresh AH or HBSS as a dilution control. The results show that LAK cells were induced to die in the presence of freshly tapped rabbit AH (Fig. 5B). Therefore, the proapoptotic factor(s) were present in AH both before and after death.

Aqueous Humor Does Not Promote Apoptosis in Ocular Tissues

Previous data had shown that AH contains a factor or factors that protect corneal endothelium from programmed cell death. We next tested the hypothesis that tissues surrounding the AC and exposed to AH, such as the iris and ciliary body, are exempt from the apoptosis-inducing factor present in AH. Transformed mouse corneal endothelial and epithelial cells and normal mouse I/CB cells were incubated in medium containing 50% AH or 50% HBSS for 24-, 48-, and 72-hour intervals. Transformed human corneal endothelial cells were also used for comparison. As shown in Figure 6A, iris/ciliary body cells were not only immune to the proapoptotic factor(s) in AH, but were significantly protected from starvation-induced cell death while incubated in 50% AH (Fig 6A). Moreover, Balb/c corneal endothelial cells incubated in the presence of AH were significantly protected from starvation-induced cell death by 48 and 72 hours (Fig. 6B). Corneal epithelial cells incubated in AH were also significantly protected from apoptosis by 48 and 72 hours (Fig. 6C). Human corneal endothelial cells behaved similarly to mouse endothelium, in that AH inhibited starvation-induced cell death (Fig. 6D). Therefore, tissues surrounding the AC were immune to the apoptosis-inducing factor(s) present in AH and were instead preserved by a dominant antiapoptotic mechanism.

Characterization of the Apoptosis-Inducing Factor(s) in Aqueous Humor

Aqueous humor contains a myriad of proteins, lipids, and peptides, any of which may be involved in the induction of cell death. Initial characterization experiments were designed to...
determine the nature (e.g., protein) and relative size of the factor(s). It was hypothesized that the apoptosis-inducing factor(s) are proteinaceous in nature. To test this hypothesis, AH was pretreated with proteinase K. To control for the effects of proteinase K, a group was included in which HBSS was subjected to the identical treatment protocol. These substrates were then used in a 24-, 48-, and 72-hour apoptosis assay using Jurkat cells. The data show a minimal level of apoptosis in cells incubated with 50% HBSS or 50% HBSS pretreated with proteinase K (Fig. 7). As before, AH induced a significant increase in the percentage of cells undergoing apoptosis. However, AH that had been pretreated with a proteolytic enzyme lost the ability to induce cell death (Fig. 7). These data show that the apoptosis-inducing factor(s) present in AH are proteinaceous in nature.

To determine a relative size for the apoptotic protein(s), AH was subjected to size fractionation by centrifugation on microconcentrators with filter membrane cutoff sizes of 10 kDa and 30 kDa. Fractions containing molecules greater than or less than 30 kDa were tested for proapoptotic activity. The results showed that proapoptotic activity segregates with the AH fraction containing molecules less than 30 kDa in size (data not shown). The AH fraction containing molecules less than 30 kDa in size was then centrifuged on a filter with a 10-kDa membrane cutoff size. Fractions containing molecules between 30 kDa and 10 kDa or less than 10 kDa were tested separately for the ability to induce cell death in LAK cells. As shown in Figure 8, the apoptosis-inducing factor(s) segregates with the AH fraction containing molecules less than 10 kDa. Together, these data show that the proapoptotic factor(s) in AH are proteinaceous and are less than 10 kDa in size. Future studies will focus on the purification and complete identification of AH-borne proteins less than 10 kDa.

**DISCUSSION**

Immune privilege in the AC of the eye is the sum of multiple protective mechanisms. These mechanisms conspire to prevent inflammatory responses that may damage the delicate ocular microanatomy. One component is the presence of numerous immunosuppressive factors in the AH. Another contributor is the elicitation of a unique immune profile (i.e., ACAID) in which the induction and expression of delayed-type hypersensitivity are profoundly suppressed. Another important mechanism is the constitutive expression of FasL on tissues surrounding the AC. FasL operates by triggering programmed cell death, or apoptosis, in cells expressing the Fas antigen. By expressing both Fas and FasL, the corneal endothelium is potentially susceptible to autoelimination through apoptotic cell death. Recent work has shown that proteins secreted by iris/ciliary body cells upregulate an antiapoptotic protein, Bcl-2, in corneal endothelial cells. The discovery of an antiapoptotic factor in AH appears to contradict the aforementioned findings in which apoptosis of immigrant inflammatory cells is necessary for the induction of ACAID and corneal grafting.

**FIGURE 4.** Aqueous humor-induced cell death in macrophages. Two Balb/c monocyte-macrophage cell lines, J774A0.1 (A) and RAW 264.7 (B), were incubated with 50% AH or HBSS for 24, 48, and 72 hours and stained for apoptotic cells by FACS. All results are expressed as the percentage of viable cells undergoing apoptosis ± SEM. *P < 0.05 for AH versus HBSS control.

**FIGURE 5.** Natural killer cells are induced to die in the presence of AH. (A) Enriched NK cells were generated from the spleens of naive Balb/c mice. Cells were incubated in medium containing 50% AH or HBSS for 24, 48, and 72 hours and stained for apoptotic cells by FACS. (B) An identical experiment was performed using freshly tapped AH. All results are expressed as the percentage of viable cells undergoing apoptosis ± SEM. *P < 0.05 for AH versus HBSS control.
acceptance. We hypothesized, however, that inflammatory cells invading the eye, such as neutrophils, macrophages and T cells, and NK cells would not be protected by the antiapoptotic factor(s) present in AH. Rather, it was postulated that AH contains a factor or factors that promote cell death in nonocular immune cells. A corollary of this hypothesis predicted that ocular tissues, such as iris/ciliary body cells, would be immune to the putative proapoptotic effect of AH.

This hypothesis was tested by incubating various immune cells in 50% rabbit AH and testing for the induction of programmed cell death. These data show that in each of the cell types tested, resting T cells, the Jurkat activated T-cell line, J774A0.1 and RAW 264.7 monocyte-macrophage cell lines, neutrophils, and NK cells, apoptosis was significantly increased after incubation with AH. The kinetics of apoptosis induction differed among cell type. In resting T cells, neutrophils, and NK cells, apoptosis was significantly enhanced by 24 hours and persisted for at least 72 hours. Activated T cells appeared unaffected until 48 hours in 50% AH. With a significant effect measured only at 72 hours, macrophages were the most resistant to the proapoptotic factor in AH. Although the exact reason remains unknown, the resistance of macrophages may reflect a requirement for resident F4/80+ macrophages to remain viable for antigen processing and presentation functions and the induction of AIAID.41,42 The susceptibility of neutrophils, T cells, and NK cells is apparently more tolerable in favor of preventing inflammation and/or NK cell-mediated attack. Future studies may reveal that resistance to the proapoptotic factor correlates with the upregulation of antiapoptotic genes, such as Bcl-2,43,44 and/or the downregulation of proapoptotic genes, such as Bax.45 If the AH contains both anti- and proapoptotic factors, then a delicate balance must exist in the sum effect of AH on each particular cell type and state of activation. It may be that susceptible cells are unaffected by the antiapoptotic factor and are therefore selected to die. Similarly, resistant cells may be either unaffected by the proapoptotic factor, or the antiapoptotic factor may dominate. Future experiments will delineate the interplay between these factors and their mechanisms of action.
Research has shown that the expression of FasL is crucial for the destruction of immigrant Fas+ inflammatory cells and is partially responsible for the induction of ACAID and corneal graft acceptance.\textsuperscript{13-16} In the case of orthotopic corneal allografts, FasL expressed on the corneal epithelium and endothelium reduces the incidence of immune rejection. Orthotopic corneal allografts prepared from FasL-defective donor mice (gld/gld mutant of C57BL/6) undergo immune rejection in 89\% to 100\% of the Balb/c recipients.\textsuperscript{15,46} By contrast, orthotopic corneal allografts prepared from C57BL/6 donors that express normal amounts of FasL are rejected in only 50\% of the Balb/c mice.\textsuperscript{15,46} However, FasL does not protect C57BL/6 corneal allografts from immune rejection if they are transplanted to heterotopic sites on Balb/c recipients.\textsuperscript{47} Thus, in addition to FasL, other factors contribute to the immune privilege of the eye and the survival of orthotopic corneal allografts. Although FasL-induced apoptosis is an important mechanism for controlling inflammation in the eye, we also suspect that, as in most immunoregulatory systems, there is redundancy, and backup mechanisms are available. It is possible that the AH-borne factor acts against nominal inflammation or against inflammatory cells that do not express functional Fas, whereas FasL acts in more robust inflammatory settings involving neutrophils and activated T cells. Moreover, the AH-borne factor may be more important in blocking inflammation from extending into the AC and may aid FasL in protecting the surface tissues immediately surrounding the AC. Because not all inflammatory cells express Fas and some cells that express Fas are resistant to FasL-induced apoptosis (e.g., recently activated T cells), we suspect that the AH-borne factor and perhaps other components of the immune privilege milieu of the eye act as ancillary anti-inflammatory elements.

An important corollary of our hypothesis predicted that ocular tissues surrounding the AC would be resistant to apoptosis induced by AH. This was tested by incubating iris/ciliary body, corneal endothelial, and corneal epithelial cell lines in AH. The results show that all ocular cell types tested are not only resistant to the proapoptotic effect of AH, but are significantly protected from starvation-induced cell death by antiapoptotic factors in the AH. These data support earlier observations in which mouse and human corneal endothelium were protected from apoptosis by the induction of Bcl-2 protein.\textsuperscript{28} The resistance of tissues lining the AC to AH-induced apoptosis may reflect immunity to the proapoptotic factor and/or a dominant effect of the antiapoptotic proteins. In this manner, the AH can preserve the integrity of ocular tissues while contributing to the prevention of intraocular inflammation and the overall maintenance of immune privilege.

We have partially characterized the proapoptotic factor(s) in AH. These data show that the proapoptotic effect is mediated by one or more proteins. Moreover, the proapoptotic effect segregates with the AH fraction containing molecules less than 10 kDa in size. More studies are necessary to characterize fully and identify the proapoptotic protein(s) contained in AH. Together with previous data, these results demonstrate a complicated and novel relationship among protein factors in the AH. Certain proteins are able to protect resident tissues through the upregulation of antiapoptotic factors (i.e., Bcl-2). Other proteins, however, function to induce programmed cell death in inflammatory cells that may irreversibly damage the eye. It remains to be determined how the proapoptotic protein(s) operate. We may find that these proteins upregulate inducers of cell death, such as Bax, or downregulate inhibitors of cell death, such as Bcl-2. Moreover, the sum effect of AH on a cell, whether resident or immigrant, may depend on any number of factors particular to each cell type. These internal characteristics must carefully regulate the dynamic relationship between these pathways. In summary, these results show a novel mechanism whereby the AH protects the integrity of intraocular tissues and preserves ocular immune privilege by the selective induction of cell death in immune cells that might otherwise damage the eye.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Proteinase K treatment destroys the proapoptotic factor(s) in AH. Aqueous humor was pre-treated with 10 \( \mu \)g/ml proteinase K to inactivate all protein (PK AH). As a control, HBSS was subjected to the identical treatment protocol (PK HBSS). Jurkat cells were collected and incubated in the presence of 50\% treated and untreated AH or HBSS for 24, 48, and 72 hours. Cells were collected and stained for apoptosis by FACS. All results are expressed as the percentage of viable cells undergoing apoptosis \pm SEM. *\( P < 0.05 \) for AH-treated versus PK AH and HBSS control.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure8.png}
\caption{Relative size of the proapoptotic factor(s). Aqueous humor was fractionated on microconcentrators with filter membrane cutoff sizes of 10 kDa and 30 kDa. LAK cells were incubated for 72 hours in the presence (50\%) of fractions containing AH molecules between 10 kDa and 30 kDa (10–30 kDa AH) and less than 10 kDa (<10 kDa AH) in size. Control wells received 50\% HBSS (HBSS) or 50\% unfractionated AH (Whole AH). Cells were collected and stained for apoptosis by FACS. All results are expressed as the percentage of viable cells undergoing apoptosis \pm SEM. *\( P < 0.05 \) for less than 10 kDa AH versus HBSS.}
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Acknowledgments

The authors thank James V. Jester and Jacquelyn Bean, University of Texas Southwestern Medical Center, Dallas, for providing rabbits and fresh rabbit aqueous humor; and Michael Bennett, University of TX Southwestern Medical Center, Dallas, for providing SCID mice.

References


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