Study on Vitreous Fibrosis Induced by Blood Cells

片 上 千 加 子
神戸大学医学部眼科学教室

要 約

硝子体出血に起因して増殖組織が形成されることは臨床的にも観察され，血液が増殖組織の発生の一因子であることが推察できる．動物の硝子体中に血液を注入して硝子体内増殖を惹起せしめた報告があるが，今回，各血液細胞が，硝子体内増殖の発生にどのような役割を果たすかを調べる目的で，家兎血液より単球，リンパ球，赤血球を分離し，各々を家兎硝子体中に注入し，硝子体内増殖組織発生の有無，重症度を注入後3週間目まで観察した．単球＋リンパ球，単球，リンパ球，赤血球の硝子体内注入は硝子体内線維増殖を惹起し，増殖の程度は，単球，リンパ球を注入したものでは，注入した細胞数に比例して増減した．硝子体内増殖発生にはリンパ球の方が単球より強力な因子であった．また，単球＋リンパ球，赤血球を注入して硝子体内増殖組織を発生させ，3週間目に硝子体を摘出し，〔^{3}H〕-glucosamine で24時間標識し，生成された〔^{3}H〕標識グリコサミノリカン（GAG）を4MGuHC1で抽出し，プロナーゼ処理し，カラムクロマトグラフィー，特異的酵素（ヒアルロニダーゼ，コンドロイチナーゼ ABC，AC）処理，薄層クロマトグラフィーの手法を用いて分析した．正常硝子体の生成する GAG は，91%がヒアルロン酸であるのに対し，増殖組織の形成された硝子体では，ヒアルロン酸の生成が約30%に減少し，かわってコンドロイチン硫酸が47～59%生成された．コンドロイチン硫酸の比率は，増殖組織の程度に比例して増加した．対象として，凍結，融解をくり返して細胞を死滅させた単球＋リンパ球を注入した硝子体では，増殖組織は形成されなかったにもかかわらず，ヒアルロン酸の生成は70%に減少し，コンドロイチン硫酸が12%生成された．これらの結果より，単球，リンパ球，赤血球の硝子体内注入が，線維芽細胞あるいは線維芽細胞類似の細胞の浸潤を惹起，または，硝子体細胞（hyalocytes）の線維芽細胞への transformation を惹起し，それらの線維芽細胞がコンドロイチン硫酸の生成に与ることが推察された．（日眼会誌 94：33—43，1990）

キーワード：硝子体内線維増殖，増殖性硝子体網膜症，グリコサミノグリカン，ヒアルロニダーゼ，コンドロイチナーゼ ABC

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Chikako Katakami
Department of Ophthalmology, School of Medicine, Kobe University

Abstract

In order to examine the individual role of each blood cell type in causing vitreous fibrosis, monocytes and/or lymphocytes, or erythrocytes were separated from rabbit blood and injected into rabbit vitreous bodies. Vitreous fibrosis was induced by intravitreal injections of the mixture of

別刷請求先：650 神戸市中央区楠町7—13—14 神戸大学医学部眼科学教室 片上千加子
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Reprint requests to: Chikako Katakami, M.D. Dept. of Ophthalmol., School of Med., Kobe Univ.
7-13-14 Kusunoki-cho, Chuō-ku, Kobe 650, Japan
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monocytes and lymphocytes, monocytes alone, lymphocytes alone, and erythrocytes. Cell density-dependent vitreous fibrosis was observed in eyes injected with monocytes and/or lymphocytes. Lymphocytes were more potent than monocytes in inducing fibrosis. Glycosaminoglycans (GAGs) synthesized by fibrotic vitreous bodies induced by the mixture of monocytes and lymphocytes, and erythrocytes were characterized. The results indicated that 91% of total GAGs synthesized by normal vitreous was hyaluronic acid (HA). In contrast, in fibrotic vitreous, the synthesis of HA was decreased to about 30% whereas that of chondroitin sulfate (CS) was increased to 47-59% of total newly synthesized GAGs. The ratio of CS against total newly synthesized GAGs in fibrotic vitreous corresponded to the grade of fibrosis. Control vitreous bodies injected with freeze-thawed monocytes and lymphocytes synthesized 70% of HA and 12% of CS although no vitreous fibrosis was observed. These results suggest that intravitreal injection of monocytes and/or lymphocytes or erythrocytes may cause the infiltration of fibroblasts or fibroblast-like cells or the transformation of hyalocytes into fibroblasts, and that these fibroblasts may be responsible for the synthesis of CS. (Acta Soc Ophthalmol Jpn 94 : 33—43, 1990)

Key words : Vitreous fibrosis, Proliferative vitreoretinopathy, Glycosaminoglycans, Hyaluronidase, Chondroitinase ABC

Introduction

Vitreous fibrosis occurs under various pathological conditions, e.g. severe penetrating ocular injury and diabetic retinopathy, and often leads to traction retinal detachment. Proliferative vitreoretinopathy is also a serious complication of retinal detachment[1]. However, the mechanism by which vitreous fibrosis occurs is not well understood. In cases of penetrating injury or diabetic retinopathy, vitreous hemorrhage can often be observed to lead to vitreous fibrosis, suggesting that blood in the vitreous is one of the factors causing vitreous fibrosis and subsequent traction retinal detachment. In cases of proliferative vitreoretinopathy, whose mechanism may differ from that of penetrating injury and diabetic retinopathy, cells responsible for proliferation were shown to be mostly derived from retinal pigment epthelial cells and retinal glial cells[2)3)]. In experimental animals, vitreous fibrosis can be induced by intravitreal injections of autologous whole blood[4)-6)], hemoglobin[7)], leukocytes[8)], platelets[9)], cultured autologous fibroblasts[10)11)], or retinal pigment epithelial cells[12)13)].

The vitreous is an avascular connective tissue composed of extracellular macromolecules, i.e., type II collagen, hyaluronic acid and glycoproteins. Its structure is maintained by a loose network of collagen fibers, which is permeated by hyaluronic acid[14)]. A small amount of glycosaminoglycans (GAGs) containing sulfated galactosamine has also been demonstrated in the adult bovine and human vitreous[15)16)].

During vitreous fibrosis, morphological and biochemical changes were reported by other investigators. The results of morphological studies indicated that the native vitreous structure is destroyed during vitreous fibrosis[17)-20)]. It was found that collagen fibrils of fibrotic vitreous are larger in diameter than those found in normal vitreous[18)]. Biochemical studies demonstrated that type I collagen is the predominant collagenous component in fibrotic vitreous induced by injection of autologous whole blood[6)], whereas normal vitreous collagen is type II.

In the present study, an individual role of each blood cell type in causing vitreous fibrosis was examined, and the glycosaminoglycans synthesized by fibrotic vitreous induced by intravitreal injection of blood cells were characterized.

Materials and Methods

Materials

[^{3}H] -Glucosamine was purchased from New England Nuclear. Eagle's minimum essential medium

was obtained from M.A. BioProducts. Sephadex G-50 (fine) was provided by Phrmacia Fine Chemicals. Chondroitinase ABC (Proteus vulgaris), chondroitinase AC (Arthrobacter aurescens), hyaluronidase (Streptomyces hyalurolyticus) and unsaturated disaccharide standards (Δdi-4S, Δdi-6S, Δdi-0S) were purchased from Seikagaku Kogyo through Miles Laboratories. Cellulose thin layer plates (20×20cm) without fluorescent indicator and X-ray film (X-omat) were purchased from Eastman Kodak. Potassium acetate, sodium borate, calcium chloride, sodium acetate, sodium chloride, pyridine, acetic acid and ethanol were purchased from Fisher Scientific Co. All other chemicals were obtained from Sigma Chemical Co.

Methods

〔Blood cells preparation〕

Twenty to 60ml of blood was drawn from adult rabbits of either sex weighing 3-4kg. Monocytes and lymphocytes were isolated from the blood using Ficoll-Isopaque technique[21]. The cell pellet containing monocytes and lymphocytes was resuspended in minimum essential medium at various concentrations. To separate monocytes from lymphocytes, the cell suspension (5×10^6cells/ml) was incubated in Petri dishes at 37℃ for 90min. The monocytes adhered to the plastic surface and the lymphocytes stayed in the suspension[22]. By differential cell count, monocytes and/or lymphocytes represented 88-95% of total cells recovered.

After removing monocytes and lymphocytes, remaining cell pellet was resuspended in MEM containing 0.4% Dextran. The erythrocytes were allowed to settle for 90 min at 37℃. The erythrocytes in the lower 1/3 portion of the test tube were collected by centrifugation and were resuspended in MEM at the concentration of 5×10^9cells/ml. All cells obtained in this preparation were erythrocytes.

〔Induction of vitreous fibrosis in rabbit eyes.〕

Adult pigmented rabbits of both sexes weighing 3-4kg were used for the experiment. The rabbits were anesthesized by combined administration of ketamine (i.m.) and sodium pentobarbital (i.v.)[6]. Drops of tetracain (1% solution) were applied topically on the eye. A cell suspension of 0.2-0.4ml containing the mixture of monocytes and lymphocytes, lymphocytes, monocytes or erythrocytes were injected into the vitreous with a 27 gauge needle through the pars plana, 0.8mm behind the limbus. The position and location of the needle in the central vitreous cavity was confirmed by visualization with the indirect ophthalmoscope before and during injection.

Twenty two eyes of 11 rabbits were injected with the mixture of monocytes and lymphocytes. Fourteen eyes of 7 rabbits received either lymphocytes or monocytes into the vitreous. Ten eyes of 5 rabbits were injected with erythrocytes. In control experiments, six eyes of 3 rabbits were injected with freeze-thawed monocytes and lymphocytes.

〔Grading of vitreous fibrosis.〕

The process of vitreous fibrosis was graded by indirect ophthalmoscopy as described by Weiss and Belkin[23]. The examiner was unaware of which cell types were injected into the vitreous. Also drawing and description were used to record the location and size of any retinal detachment. The following grading system was used : 0=no vitreous fibrosis ; I=fine wisps of fibrosis ; II=heavier wisps of fibrosis ; III_1=thin band (s) of fibrosis ; III_2=marked thick band (s) of fibrosis ; IV_1=thin band (s) of fibrosis with traction retinal detachment ; IV_2=thick band (s) with traction retinal detachment.

〔Labeling of tissue.〕

Twenty four eyes of 12 rabbits including 6 eyes injected with monocytes and lymphocytes (4.2×10^6 cells/eye), 6 eyes injected with erythrocytes (10^9cells/eye), 6 eyes injected with freeze-thawed monocytes and lymphocytes (4.2×10^6cells/eye) and 6 normal eyes were used for biochemical analysis. The rabbits were sacrificed after 3 weeks by sodium pentobarbital overdose. The vitreous was removed from the enucleated eyes by a circumferential equatorial scleral incision. Care was taken to avoid contamination of adjacent tissues. Each indivisual vitreous was then labeled with 〔^{3}H〕 glucosamine (40μCi/ml) in 3ml of MEM supplemented with gentamicin at 37℃ for 24h. After incubation, the samples were then lyophilized without separating the tissues from the medium.

〔Characterization of glycosaminoglycans.〕

The lyophilized vitreous was extracted in 3ml of a solution containing 4M guanidium hydrochloride, 10mM EDTA, 10mM n-ethylmaleimide, 1mM phenylmethane sulfonyl fluoride and 50mM Tris-HCl buffer, pH 8.0 as described by Shinomura, et al.[24]. The extract was then precipitated with 3 volumes of 95% ethanol containing 0.3% (w/v) potassium acetate. The precipitate was washed with water, reprecipitated with cold ethanol three times and dialyzed against 20mM $CaCl_2$, 0.1M Borate buffer, pH 8.5. The samples were then digested with 200μg of pronase (protease type xiv, Sigma) at 60℃ for 24h and subjected to gel filtration column chromatography of Sephadex G-50 (1.6×90cm) in 0.2M pyridine-acetate, pH 5.0[25]. Samples pooled from the peaks of the column were lyophilized. The lyophilized residues were reconstituted in various buffers as indicated below, and subjected to specific enzyme digestion by streptomyces hyaluronidase (500units/ml), chondroitinase ABC (10units/ml), or chondroitinase AC (10units/ml). Hyaluronidase treatment was carried out in 75mM sodium acetate buffer, pH 6.0, at 60℃ for 5h[26]. Chondroitinase ABC and AC digestion was carried out in 50mM Tris-HCl buffer, pH 8.0 at 37℃ for 16h[25]. Each enzyme digest was further fractionated with a G-50 column (1×30cm) to estimate the amount of component (s) which was sensitive to the specific enzymes. The resulting disaccharides of chondroitinase ABC digest were further analyzed by thin layer chromatography as described by Mason et al.[27].

Results

〔Vitreous fibrosis induced by blood cells.〕

Twenty two eyes received an intravitreal injection of monocytes and lymphocytes. All eight eyes receiving a dose of 6.2×10^6cells or greater in the vitreous cavity developed vitreous fibrosis and traction retinal detachment (Table 1). The other fourteen eyes receiving a dose of between 0.5×10^6 and 4.2×10^6 cells into the vitreous cavity developed no traction retinal detachment (Table 1).

Six of eight eyes receiving intravitreal injection of lymphocytes developed traction retinal detachment. These six eyes received 1.4×10^6cells per eyes or greater. All eight eyes receiving the intravitreal

Table 1 Cell density-dependent vitreous fibrosis with intravitreal injection of mixture of monocytes and lymphocytes.

Exp. No.	No. of Eyes	Cell No./Eye	Fibrosis*		
			1 week	2 weeks	3 weeks
I.	6	10.0×10^6	III$_2$	IV$_2$	IV$_2$
II.	2	6.2×10^6	III$_2$	IV$_2$	IV$_2$
III.	6	4.2×10^6	III$_1$	III$_1$	III$_2$
IV.	4	2.0×10^6	II	III$_1$	II
V.	2	1.0×10^6	III$_1$	III$_1$	III$_1$
VI.	2	0.5×10^6	II	II	I

*The grade of vitreous fibrosis is the average grade of eyes indicated.

Table 2 Cell density-dependent vitreous fibrosis with intravitreal injection of monocytes or lymphocytes.

Exp. No.	No. of Eyes	Cell Type	Cell No./Eye	Fibrosis*		
				1 week	2 weeks	3 weeks
I.	2	lymphocytes	2.4×10^6	III$_2$	III$_2$	IV$_1$
II.	4	lymphocytes	1.4×10^6	II	III$_1$	IV$_1$
III.	2	lymphocytes	0.4×10^6	III$_1$	III$_1$	III$_1$
IV.	2	monocytes	2.5×10^6	II	II	II
V.	4	monocytes	2.0×10^6	III$_1$	II	II

*The grade of vitreous fibrosis is the average grade of eyes indicated.

lymphocytes did develop vitreous fibrosis (Table 2). All six eyes receiving intravitreal monocytes developed vitreous fibrosis without retinal detachment (Table 2).

All ten eyes receiving erythrocytes into the vitreous developed vitreous fibrosis with thick band formation. Eight of ten eyes developed vitreous fibrosis with traction retinal detachment (Table 3).

No vitreous fibrosis was observed in control eyes which were injected with freeze-thawed monocytes and lymphocytes.

〔GAG synthesized by normal vitreous.〕

Vitreous removed from normal eye was labeled with 〔^{3}H〕-glucosamine at 37℃ for 24h. The newly synthesized GAG was isolated as described under Methods. Approximately 80% of the ^{3}H-labeled GAG was recovered near the void volume of G-50 column (Kav=0.07) as shown in Fig. 1a. Of this material 91% was degraded by hyaluronidase (Fig. 1b, Table 4). The hyaluronidase degraded material was eluted from G-50 column at a position having a Kav value of 0.63, suggesting a form of tetra- or hexasaccharide. Another aliquot of the material eluting near the void volume was digested with chondroitinase ABC and the resulting disaccharides were further analyzed by thin-layer chromatography. As demonstrated in Fig. 3, the material degraded by chodroitinase ABC was recovered as Δdi-HA.

〔GAG synthesized by fibrotic vitreous.〕

Fibrotic vitreous obtained from eyes injected with monocytes and lymphocytes (4.2×10^6 cells in 0.2ml

Table 3 Vitreous fibrosis with intravitreal injection of erythrocytes.

Exp. No.	No. of Eyes	Cell No./Eye	Fibrosis*		
			1 week	2 weeks	3 weeks
I.	2	2×10^9	III$_2$	III$_2$	IV$_2$
II.	2	10^9	III$_1$	III$_2$	IV$_2$
III.	2	10^9	II	III$_1$	III$_2$
IV.	4	10^9	III$_1$	III$_2$	IV$_2$

*The grade of vitreous fibrosis is the average grade of eyes indicated.

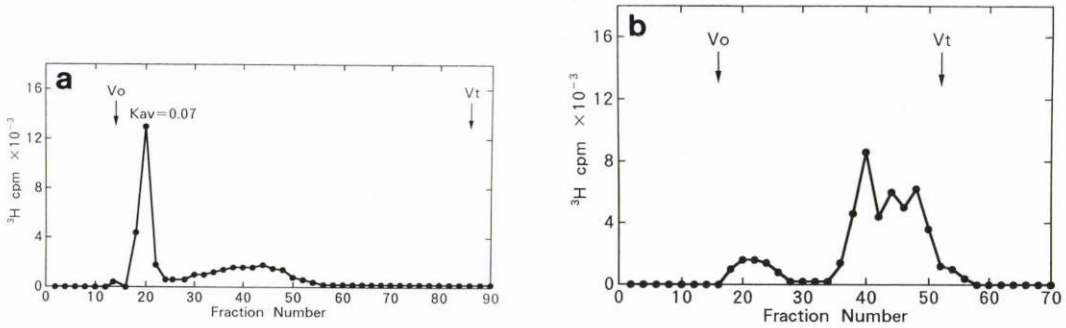

Fig. 1 a; Glycosaminoglycan synthesized by normal vitreous. Vitreous obtained from normal eye was labeled with 〔^{3}H〕-glucosamine at 37℃ for 24h. The newly synthesized GAG was fractionated on Sephadex G-50 column (1.6×90 cm) at flow rate of 9ml/h. Two ml were collected in each fraction. Aliquots of 0.1ml from the fractions were counted in 10ml of Scintiverse (Fisher). b; Hyaluronidase digestion of newly synthesized GAG by normal vitreous. One-half of the pooled fractions eluted near the void volume from Sephadex G-50 column (Fig. 1a) was digested with Streptomyces hyaluronidase as described under Methods. The digest was then applied to another Sephadex G-50 column (1×30cm) at a flow rate of 9ml/h and fractions of 0.4ml were collected. Aliquots of 0.1ml were counted.

Table 4 Glycosaminoglycan composition of normal and fibrotic vitreous

Vitreous Sample	Cells Injected	Grade of* Vitreous Fibrosis	Glycosaminoglycans** (% of Total)		
			HA***	CS****	uncharacterized
Normal			91.0	N.D.	9.0
Control	Freeze-thawed monocytes and lymphocytes	0	69.5	12.4	18.1
Fibrotic	Living monocytes and lymphocytes	III_2	30.9	47.1	22.0
Fibrotic	Erythrocytes	IV_2	26.0	59.0	15.0

N.D. not detectable.

*The grade of vitreous fibrosis is the average grade of eyes in each group.

**Numbers are the average of 6 eyes in each group.

***Material degraded by streptomyces hyaluronidase, % of total ^{3}H-labeled GAGs.

****Material resistant to streptomyces hyaluronidase, but sensitive to chondroitinase AC, % of total ^{3}H-labeled GAGs.

MEM) or erythrocytes (0.2ml of cell suspension prepared as described in Methods) were labeled with [^{3}H]-glucosamine as described above. When the radioactively labeled GAG was fractinated by a Sephadex G-50 column, two peaks containing radioactivity were recovered (Fig. 2a, 2d). Kav values of the peaks of the fibrotic vitreous induced by monocytes and lymphocytes (M+L) were 0.07 and 0.41(Fig. 2a), while those of the fibrotic vitreous induced by erythrocytes (E) were 0.1 and 0.36(Fig. 2d). The material eluted at the first peak was treated with hyaluronidase. Only 30% (M+L), and 26% (E) of the total radioactivity of the first peak were hyaluronidase sensitive (Fig. 2b, 2e). To examine whether the digestion was complete, the hyaluronidase-resistant material was treated with hyaluronidase again and no further degradation was observed. The hyaluronidase resistant material was digested with chondroitinase AC. The results indicated that 70% (M+L), and 81% (E) of the hyaluronidase-resistant material were degraded by chondroitinase AC (Fig. 2c, 2f). Another aliquot of the material recovered near the void volume of G-50 column was digested with chondroitinase ABC and the resulting disaccharides were further analyzed by thin layer chromatography. It was found that the newly synthesized GAGs contained Δdi-6S, Δdi-4S, Δdi-0S and Δdi-HA (Fig. 3). As indicated in Table 4, the fibrotic vitreous induced by monocytes and lymphocytes contained 31% HA, 47% chondroitin sulfate (CS) and 22% uncharacterized GAG, whereas fibrotic vitreous induced by erythrocytes contained 26% HA, 59% CS and 15% uncharacterized GAG. In a control experiment, eyes injected with freeze-thawed monocytes and lymphocytes did not develop fibrosis. Vitreous from these eyes were labeled and analyzed as described above. It was found that 70% of the newly synthesized GAG was HA (Fig. 2h). Approximately 50% of the hyaluronidase-resistant material was further degraded by chondroitinase AC (Fig. 2i). Thin layer chromatography analysis of disacchrides derived from chondroitinase ABC digest revealed that the vitreous contained only Δdi-6S and Δdi-HA (Fig. 3). As shown in Table 4, vitreous from the control eyes synthesized 70% HA, 12% CS and 18% uncharacterized GAG.

Discussion

Clinically vitreous hemorrhage can often be observed to lead to vitreous fibrosis, suggesting that blood in the vitreous is one of factors causing vitreous fibrosis. Experimentally, intravitreal injections of whole blood, hemoglobin, leukocytes, and platelets can induce vitreous fibrosis[4)-9)]. However, an individual role of each blood cell type in causing vitreous fibrosis remains unknown. In our separate experiment, it was shown that intravitreal injections of polymorphonuclear leukocytes and plasma do not induce vitreous fibrosis.

In the present study it was demonstrated that vitreous fibrosis can be induced by intravitreal

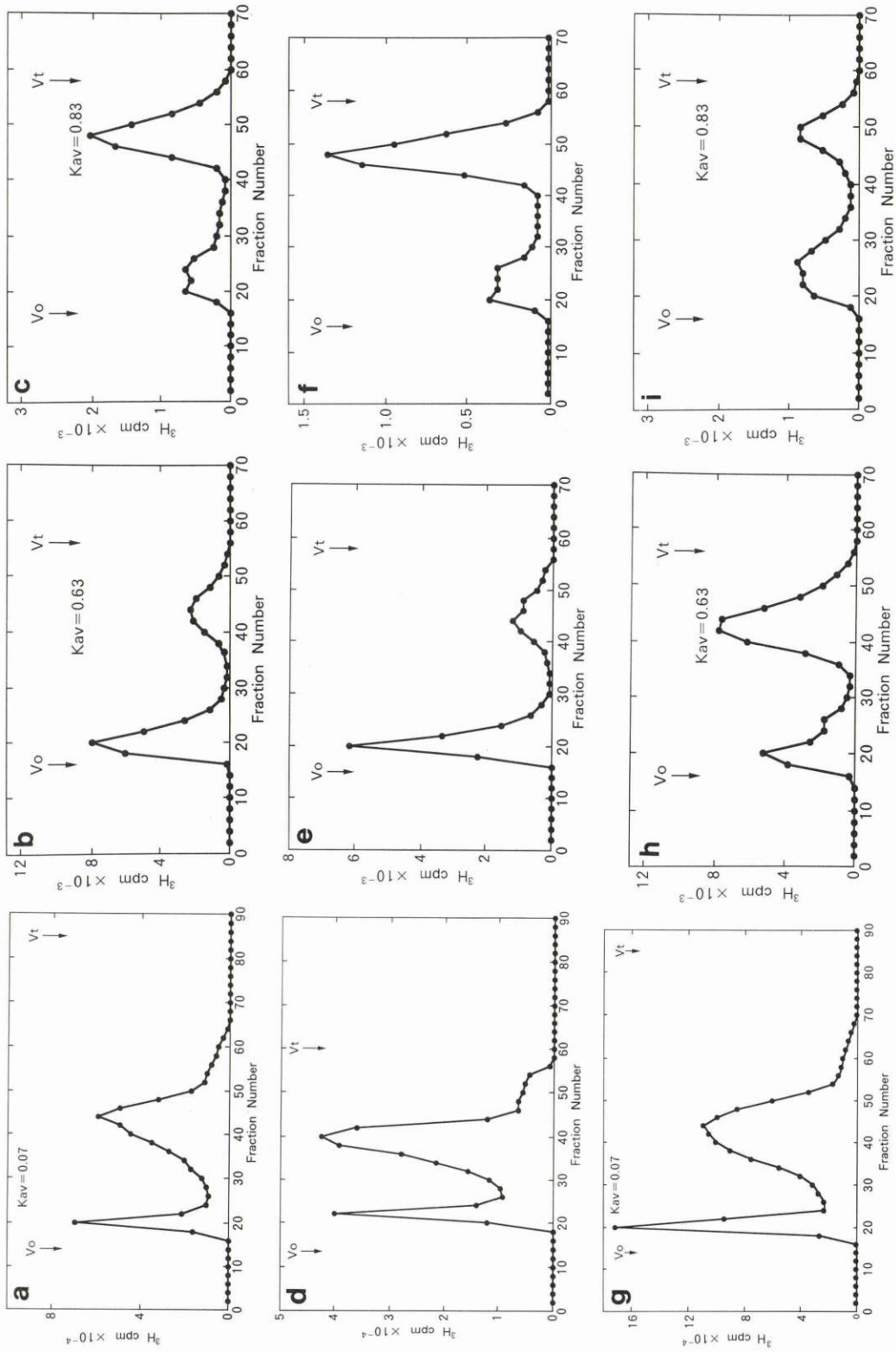

Fig. 2 Glycosaminoglycan synthesized by fibrotic vitreous induced by intravitreal injection of monocytes and lymphocytes (a, b, c), erythrocytes (d, e, f) and control vitreous injected with freeze-thawed monocytes and lymphocytes (g, h, i). GAG synthesized by fibrotic and control vitreous was fractionated as described in Fig. 1. The pooled fractions eluted near the void volume from Sephadex G-50 column (a, d, g) were digested with Streptomyces hyaluronidase and applied to another Sephadex G-50 column (b, e, h). The hyaluronidase-resistant materials which were eluted near the void volume (b, e, h) were digested with chondroitinase AC and applied to Sephadex G-50 column (c, f, i).

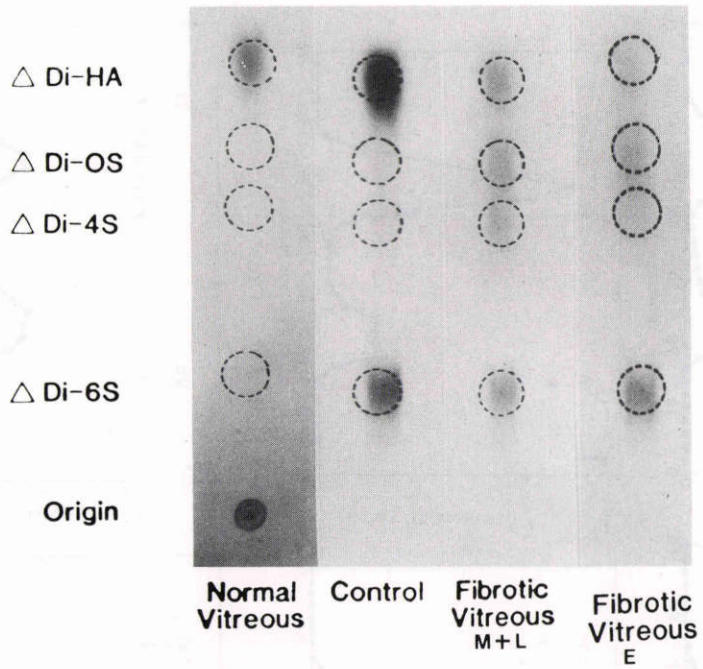

Fig. 3 Thin-layer chromatography of chondroitinase ABC digestion of newly synthesized GAG. One-half of the pooled fractions eluted near the void volume from Sephadex G-50 column (See Fig. 1a, 2a, 2d, 2g) was lyophilyzed and reconstituted in 100μl of a buffer solution containing chondroitinase ABC (10 units/ml). The enzyme digestion was carried out at 37℃ for 16h. The digest was then applied to a Sephadex G-50 column (1×30cm). The material eluted near the total volume (Kav=0.83) was pooled and lyophilized. The residues were then reconstituted in 20μl of a solution containing 50μg each of Δdi-HA, Δdi-OS, Δdi-4S, and Δdi-6S. The samples were then subjected to thin-layer chromatography as described under Methods. The positions of the disaccharides in thin-layer were observed under UV light and marked on thin-layer chromatography plates prior to the preparation of fluorograms. The circles indicated the positions of respective disaccharides observed on thin-layer chromatography plates.

injections of the mixure of monocytes and lymphocytes, monocytes alone, lymphocytes alone and erythrocytes, and that the degree of vitreous fibrosis induced by monocytes and/or lymphocytes was parallel with the number of cells injected. It should also be noted that lymphocytes are more potent than monocytes in inducing vitreous fibrosis. This may in part explain the observation that more cells were needed to cause vitreous fibrosis when the mixture of monocytes and lymphocytes were injected into the vitreous. However, it is not known whether lymphocytes and monocytes are antagonistic to each other in inducing vitreous fibrosis.

In this experiment, selected eyes with vitreous fibrosis were subjected to histological examination which revealed increased collagen fibers in the vitreous associated with infitration of inflammatory cells and fibroblasts.

Although the mechanism by which monocytes and/or lymphocytes cause vitreous fibrosis remains unknown, several explanations are possible. Monocytes injected into vitreous may differentiate into macrophages and/or macrophages may migrate from surrounding tissues in response to the trauma.

Macrophages have been shown to secrete certain factors which are chemotactic[28] and stimulate proliferation of various cells[29)-31)]. On the other hand, soluble factors produced by lymphocytes (lymphokines) also have been shown to stimulate fibroblast migration and proliferation[32)-34)]. It is possible that in response to these factors, cells in surrounding ocular tissues may migrate into the vitreous and transform into fibroblasts.

The mechanism by which erythrocytes cause vitreous fibrosis also remains unknown. Regnault[7] investigated the fate of erythrocytes after intravitreal injection of whole blood and emphasized the toxicity of hemoglobin and its degradation products on retinal cells. It is possible that macrophages may invade into vitreous in response to the erythrocytes injection which leads to the release of hemoglobin in the vitreous. Macrophages may secrete factors, as described above, which cause fibroblasts migration from surrounding ocular tissues and subsequent vitreous fibrosis.

It has been reported that hyalocytes which are very seldom engaged in mitotic activities under normal conditions can be induced to proliferate in response to photocoagulation of the retina[35] or to intravitreal injection of hemoglobin[36]. Therefore, it is also possible that hyalocytes themselves may transform into fibroblasts in response to factors secreted by other cells, e.g. macrophages or to intravitreal hemoglobin. However, these hypotheses should be further investigated.

The major GAG component in normal vitreous is HA[14)37)]. Several lines of evidence suggest that hyalocytes synthesize HA[38)-40)]. It has also been suggested that cells in surrounding tissues, such as the retina or the ciliary body may also synthesize vitreous HA[37]. Under certain pathological conditions, e.g., penetrating eye injury, the content of vitreous may change. It was reported that the HA concentration decreases in the early period after the injection of blood into rabbit vitreous and after a period of 6 weeks to 3 months it increases again in these eyes which contain significant amounts of fibroblastic scar tissue[17)18)]. These investigators suggest that the reformation of HA in those eyes is due to the proliferating scar tissue rather than to restoration of native vitreous HA.

In the present study, GAGs synthesized by normal and fibrotic vitreous were characterized. The results of in vitro pulse-labeling studies demonstrated that most of the GAG synthesized by normal vitreous was HA. In contrast, in the fibrotic vitreous induced by intravitreal injection of the mixture of monocytes and lymphocytes (M+L) or erythrocytes (E), the synthesis of HA decreased markedly, whereas the synthesis of CS increased to about 47% (M+L) and 59% (E) of the total newly synthesized GAGs. Although no fibrosis was observed, the amount of HA also decreased slightly in the control vitreous which was injected with freeze-thawed monocytes and lymphocytes. However, only a small percentage of newly synthesized GAGs was recovered as CS. In addition, the elution profiles on the Sephadex G-50 column chromatography of both control and fibrotic vitreous showed a distinct second peak in an included position. These fractions were resistant to both hyaluronidase and chondroitinase ABC, and their chemical composition remains unknown.

It is of interest that in fibrotic vitreous the percentage of CS against newly synthesized GAGs was parallel with the grade of vitreous fibrosis. The cell type responsible for the synthesis of CS is not known. It is unlikely that monocytes and lymphocytes injected into the vitreous are responsible for CS synthesis, since these elements would probably have migrated out of the vitreous or would have been removed by the host response during the three weeks after injection. It had been reported that fibroblast-like cells were associated with collagen fibers in fibrotic vitreous induced by intravitreal injection of whole blood[41]. It was demonstated that type I collagen, a product synthesized by fibroblasts, is the major collagenous component in fibrotic vitreous[6]. In addition, it has been shown that fibroblasts are capable of synthesizing CS[42]. Therefore, it is very likely that fibroblasts or fibroblast-like cells are responsible for the synthesis of CS in fibrotic vitreous.

It is of particular interest that the intravitreal injection of freeze-thawed monocytes and lymphocytes also induced a slight increase in the synthesis of CS, even though no apparent fibrosis was observed. The explanation of this is that the injected freeze-thawed monocytes and lymphocytes may cause a mild host inflamation and/or immune response and subsequently the infiltration of fibroblasts or fibroblast-like cells

or the transformation of hyalocytes into fibroblasts. Since intravitreal injection of living cells caused more severe reaction, it was suggested that living cells play active role in inducing vitreous fibrosis.

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