Detection of Cell Adhesion Molecules in Lens Epithelial Cells of Human Cataracts

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Purpose. To detect the expression of cell adhesion molecules (CAMs), including β integrins, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, leukocyte–endothelial cell adhesion molecule-2, E-cadherin, and CD44, in cultured lens epithelial cells (LECs) of human cataracts. To show that LECs attach to cells or extracellular matrix components by the detected adhesion molecules.

Methods. A circular section of the anterior capsule with attached LECs obtained by anterior capsulotomy during cataract surgery was cultured in a well of an eight-chamber slide. The LEC immediately after surgery and the cell outgrowth beyond the capsular margin at 2 weeks of culture were observed after the culture was stained immunohistochemically. Functional assays of LEC growth on collagen- or laminin-coated plates were performed in the presence and absence of the antibody blocking the detected adhesion molecules.

Results. β_1 integrin, ICAM-1, and CD44 were detected in both the original specimens and the cultured cells. When the antihuman anti-β_1 integrin monoclonal antibody (mAb), anti-ICAM-1 mAb, or anti-CD44 mAb was added at 10 μg/ml to the incubation medium, LEC migration and proliferation were inhibited significantly on the collagen- or laminin-coated plates. When the mAb blocking these three CAMs were added each at 1 μg/ml, LEC proliferation also were inhibited.

Conclusions. β_1 integrin, ICAM-1, and CD44 are all involved in LEC attachment and growth on collagen and laminin in vitro. It can be assumed that these CAMs are involved in adhesion of LECs to extracellular matrix components of the lens capsule. Understanding the characteristics of the adhesion molecules in LEC may lead to the development of a new approach to inhibit secondary cataract formation. Invest Ophthalmol Vis Sci. 1997;38:579–585.

Cell adhesion molecules (CAMs) play a central role in the cellular adhesion mechanisms characterized by cell-to-cell and cell-to-extracellular matrix (ECM) interactions. They are known to serve as adhesion receptors as well as biologic signal transducers in these interactions. The presence of various adhesion molecules has widely been reported in normal or inflamed human cornea,[1–4] iris,[5] uvea,[6–8] retina,[9] epiretinal membrane,[10,11] and diabetic epiretinal membranes.[12] They were found in the serum of patients with uveitis.[13]

After cataract surgery, residual lens epithelial cells (LECs) proliferate in the defective lens capsule and migrate onto the posterior capsule after surgery. The LECs undergo fibrous pseudometaplasia at the capsulotomy site as an expression of wound healing or when they come in contact with the implanted intraocular lens (IOL). Through this process, the blood–aqueous barrier can be disrupted.[14] A model system that parallels this is LECs in culture that proliferate and undergo a fibroblast-like cell change in the culture plate well and consequently produce various cytokines such as interleukin-1 (IL-1),[15] interleukin-6,[16] transforming growth factor-β[17] and b-fibroblast growth factor,[18] and prostaglandin E_2.[15] We have proposed that this interrelation between LEC and IOL should be considered as a criterion for evaluating the biocompatibility of an IOL. Adhesion molecules in the LEC may be involved and play an important role in all these processes. Regarding the expression of CAM in LEC, to our knowledge, there is one report by Menko and Philip[19] that

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β_1 integrin was noted in the embryonic lens. Here we have attempted to characterize the expression of β_1 integrins and some other families of adhesion molecules in the cultured human cataract LEC by the immunohistochemical technique. In addition, we performed functional assays on LECs growing on collagen- or laminin-coated plates in the presence and absence of blocking antibodies against the detected CAM to confirm that the LECs attach to the cell or extracellular matrix components through the CAM.

MATERIALS AND METHODS

This study was approved by the Institutional Ethical Review Committee at the Jinshikai Medical Foundation, Nishi Eye Hospital, Osaka, Japan. All procedures in this study were performed in full accordance with the tenets of the Declaration of Helsinki.

Culture of Human Cataract Lens Epithelial Cells

Human cataract LECs were cultured as described previously.[15] Briefly, a circular section of the anterior capsule with LECs attached was obtained by capsulotomy during cataract surgery and cultured directly without dispersion of the cells. It was placed immediately into a chamber of an eight-chamber slide containing 0.35 ml of Eagle's minimum essential medium containing 10% fetal calf serum, penicillin G (100 U/ml), and streptomycin sulfate (100 μg/ml). The specimen then was cultured in 100% humidity at 37°C in a 5% carbon dioxide atmosphere for 2 weeks. The culture medium was replaced every 7 days.

Immunohistochemical Detection of Adhesion Molecules

Cultured Lens Epithelial Cells. After the incubation medium was removed at 2 weeks of culture, the culture was washed twice with calcium- and magnesium-free phosphate-buffered saline (-), while the chamber slide containing the LECs was placed on ice. The LEC specimens then were stained for β_1, β_2, and β_3 integrins, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), leukocyte–endothelial cell adhesion molecule-2, E-cadherin, and CD44. The specimens at 2 weeks of culture were used, because LECs were growing, but none of the LEC had reached the well wall, so there was no contact inhibition.

The LECs were incubated with 5% goat serum (Cappel, Durham, NC) for 60 minutes at room temperature to block nonspecific binding. After intensive rinsing with phosphate-buffered saline containing 0.05% polysolvate, the culture then was stained with mouse antihuman β_1, β_2, and β_3 integrin monoclonal antibodies (mAb) (diluted 1:300, 1:100, and 1:100, respectively, with phosphate-buffered saline [−] containing 1% bovine serum albumin and 0.1% azide sodium; Sumitomo Denko, Yokohama, Osaka, Japan), ICAM-1 mAb (diluted 1:100; Genzyme, Cambridge, MA), VCAM-1 mAb (diluted 1:100; Genzyme), LECAM-2 mAb (diluted 1:10; Seikagaku Industries, Osaka, Japan), E-cadherin mAb (diluted 1:100 with 20 mM Tris buffer solution, 1% bovine serum albumin, 0.1% azide sodium, and 10 mM calcium chloride; Takara Breweries, Kyoto, Japan), or CD44 mAb (diluted 1:100; Cosmo Bio, Tokyo, Japan) as the primary antibody for 60 minutes at room temperature. After intensive rinsing, fluorescein isothiocyanate-conjugated goat antimouse immunoglobulin G (H + L) (diluted 1:300; ZYMED, San Francisco, CA) was used as the secondary antibody fluorescein isothiocyanate-conjugated mouse immunoglobulin G (Dako, Glostrup, Denmark) served as the negative control specimen.

In Situ Lens Epithelial Cells. Each of the specimens taken immediately after surgery was placed on a silane (amino-propyl-triethoxy silane)-coated slide glass with the LEC facing the slide and left for 2 minutes. The capsule of the specimen was removed, leaving the cells on the slide, which then were stained. Five to seven specimens were used for the detection of each CAM for both cultured and in situ LECs.

Functional Assays of Lens Epithelial Cells to Collagen- and Laminin-Coated Plates

The specimen was incubated on a collagen IV- or laminin-coated plate for 2 weeks. At each medium change, mouse antihuman anti-integrin mAb (Sumitomo Denko) was added to the incubation medium at the concentration of 0.01, 0.1, 1, or 10 μg/ml. Mouse antihuman anti-ICAM-1 mAb (Genzyme) or anti-CD44 mAb (Cosmo Bio, Tokyo, Japan) was added at a concentration of 0.1, 1, or 10 μg/ml. In one experiment, a combination of all three antibodies (1 μg/ml each) was added at the medium change. As the control specimen, immunoglobulin G1 (Bethyl Lab, Montgomery, TX) was used. Nonadherent cells were removed by aspiration at each medium change. Five to seven specimens were used for each adhesion molecule at each concentration. The number of attached viable cells was counted at the end of culture as reported previously.[15]

Statistical Analyses

The statistical analyses of the results in the functional assays were performed by Scheffe's multiple comparison after the Kruskal–Wallis test.

RESULTS

Detection of Adhesion Molecules

Definite positive expressions of β_1 integrin ($n = 5$ and $n = 7$, immediately after surgery and at 2 weeks of

culture, respectively), ICAM-1 ($n = 5$ and $n = 5$), and CD44 ($n = 6$ and $n = 5$) were observed (Figs. 1, 2, and 3) immediately after surgery and at 2 weeks of culture in all the cells of each specimen. Numerous small, round adhesion plaques on the cell were stained brightly by the antibodies for β_1 integrin, ICAM-1, and CD44. Table 1 summarizes the data from these experiments. Of the specimens taken immediately after surgery, 80% showed strong staining for CD44 and 20% stained moderately. After 2 weeks of culture, 60% exhibited strong CD44 staining, whereas 40% were moderately positive for this antigen. The control specimens did not show any positive staining. The monoclonal antibodies used are specific to each of the detected human CAM: β_1 integrin mAb binds to α- and β-chains; CD44 recognizes a transmembrane molecule with extensive glycosylation (O-linked and N-linked).

In the specimens stained β_2 ($n = 5$, $n = 6$) and β_3 ($n = 5$, $n = 6$) integrins, VCAM-1 ($n = 5$, $n = 5$) and LECAM-2 ($n = 5$, $n = 6$), E-cadherin ($n = 5$, $n = 6$), no cells were stained positively in either the specimens obtained both immediately after surgery or those at 2 weeks of culture.

Functional Assay of Lens Epithelial Cells to Collagen- or Laminin-Coated Plates

Cell growth was inhibited significantly by anti-β_1 integrin mAb at 1 μg/ml ($P < 0.01$) and 10 μg/ml ($P < 0.01$), anti-ICAM-1 mAb at 1 μg/ml ($P < 0.05$) and 10 μg/ml ($P < 0.01$), and anti-CD44 mAb at 10 μg/ml ($P < 0.01$) on the collagen IV-coated well. Both were inhibited significantly on the laminin-coated well by anti-β_1 integrin mAb at 0.1 μg/ml ($P < 0.05$), 1 μg/ml ($P < 0.01$), and 10 μg/ml ($P < 0.01$) dose dependently; by anti-ICAM-1 mAb at 1 μg/ml ($P < 0.01$) and 10 μg/ml ($P < 0.01$); and anti-CD44 mAb at 10 μg/ml ($P < 0.01$). The combined addition of the three mAbs together, each at 1 μg/ml, inhibited significantly LEC migration and proliferation both on the collagen IV- and laminin-coated wells (Figs. 4, 5, and 6).

DISCUSSION

The results show that human cataract LEC express β_1 integrin, ICAM-1, and CD44 in the specimens that were stained immediately after surgery. This suggests that these adhesion molecules are expressed in situ, being participants in the interactions between LEC and LEC and between LEC and the capsule as well. These CAM also could be detected in the specimens at 2 weeks of culture in vitro. VCAM-1 and E-cadherin were not detectable either immediately after surgery or at 2 weeks of culture. In our previous report,[20] there was positive staining for β_1 integrin and VCAM-1, but ICAM-1 was not present at 2 weeks of culture. The difference in the results may have been caused by the specimen fixation with 95% ethanol in the previous report, which could alter the cellular surface. The specimen may have been stained nonspecifically or the CAM may have been denatured. In contrast, in the current study, the specimens were stained without any fixation procedure, which ensured specific staining with the corresponding monoclonal antibody.

Results from the functional assays of the β_1 integrin and CD44 using collagen IV- or laminin-coated wells showed clearly that these adhesion molecules are involved in cell adhesion and growth on ECM in vitro. Fibronectin, collagen, or laminin on the cell surface is a ligand belonging to the integrin family or CD44. Blocking of ICAM-1 inhibited significantly cell growth on these ECMs, although they do not belong to the ligand family of ICAM-1. This suggests the blocking of ICAM-1 may inhibit cell adhesion through other pathways.

Our current findings suggest that the detected CAM may serve directly or indirectly in the attachment of the LEC to the underlying basement membrane (i.e., lens capsule) because collagen and laminin are major components of the lens capsule's ECM. Also, in LEC migration and the attachment onto the posterior capsule in the defective lens capsule after cataract surgery, such molecules presumably are involved in the formation and disruption of cell-to-cell and cell-to-posterior capsule interactions.

Olivero and Furcht[21] reported that fibronectin in addition to collagen IV and laminin promoted the adhesion and migration of rabbit LEC. We did not investigate fibronectin into our study, because normally it is not present in the adult lens. However, fibronectin plays a role in the embryonic development of the lens.[21] Therefore, it would be of interest to determine in further studies how human cataract LECs respond to fibronectin in the presence and absence of blocking antibodies, because the authors cited above assumed that the introduction of fibronectin into the eye after cataract surgery may play a critical role in the posterior migration of LECs.

Our results may have clinical implications for the development of new approaches to inhibit posterior capsule opacification and postoperative inflammation caused specifically by residual LECs. When residual LECs come in contact with the IOL after surgery, these cells are induced to undergo fibrous metaplasia and produce prostaglandin E_2, resulting in capsular fibrosis and blood–aqueous barrier disruption.[14] In this process, the IOL can be regarded to act as an ECM. We speculate that residual LECs may receive a pathologic signal from the IOL through such adhesion molecules, when the IOL functions as an ECM, because a proteinaceous coating around the IOL was found after

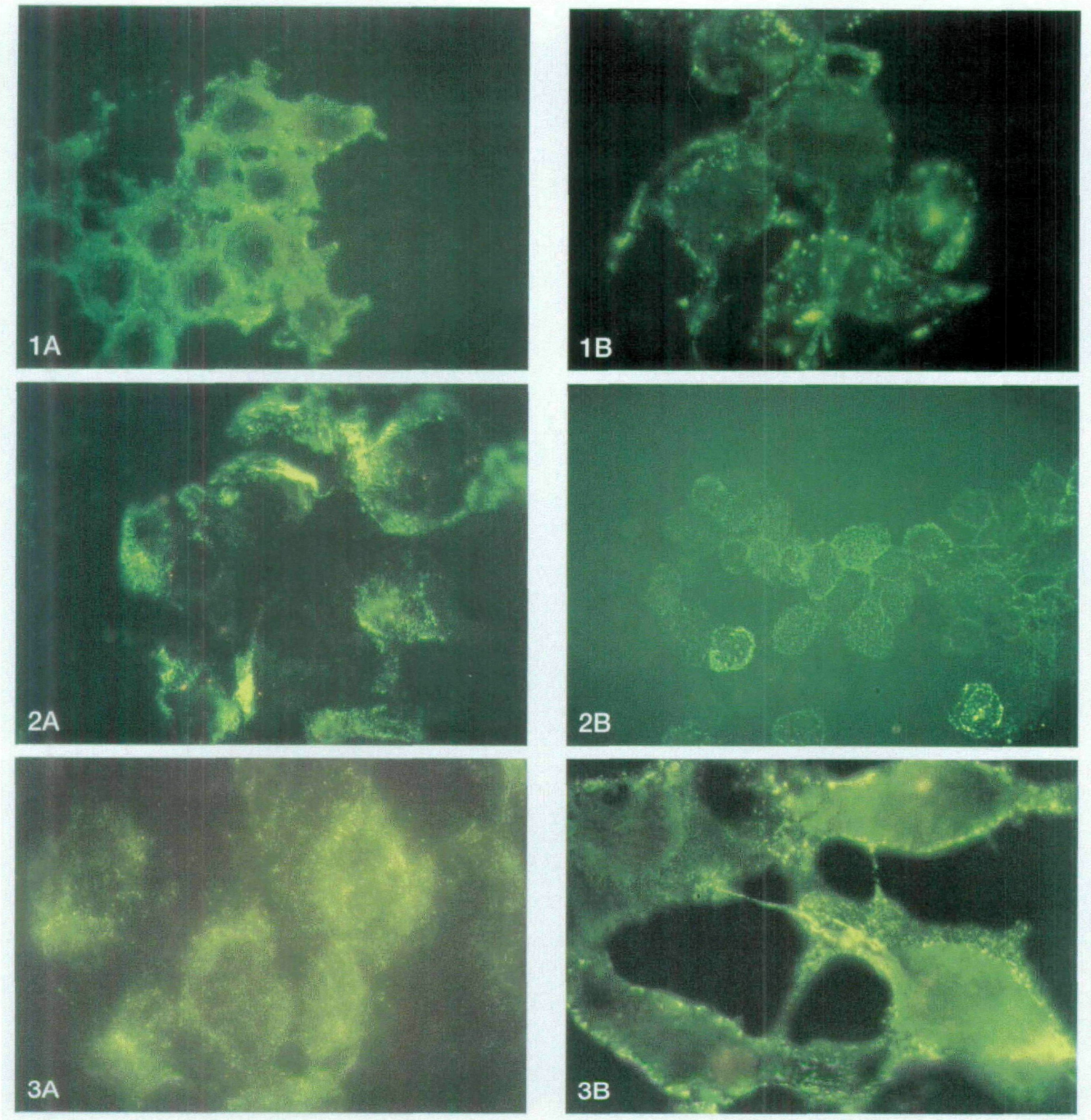

FIGURE 1. Expression of β_1 integrin in situ immediately after surgery (**A**) and at 2 weeks of culture (**B**) in human cataract lens epithelial cells. Lens epithelial cells show immunoreactivity for β_1 integrin. Magnifications, ×400 and ×1000 for **A** and **B**, respectively.

FIGURE 2. Expression of intercellular adhesion molecule-1 in situ immediately after surgery (**A**) and at 2 weeks of culture (**B**) in human cataract lens epithelial cells. Lens epithelial cells show immunoreactivity for intercellular adhesion molecule-1. Magnifications, ×1000 and ×200 for **A** and **B**, respectively.

FIGURE 3. Expression of E-cadherin in situ immediately after surgery (**A**) and at 2 weeks of culture (**B**) in human cataract lens epithelial cells. Lens epithelial cells show immunoreactivity for E-cadherin. Magnification, ×1000.

TABLE 1. Expression of Adhesion Molecules by Lens Epithelial Cells of Human Cataracts

	Before Culture		*2 Weeks of Culture*	
	Number of Donors	*Staining*	*Number of Donors*	*Staining*
β_1 Integrin	5	5 (strong)	7	7 (strong)
β_2 Integrin	5	0	6	0
β_3 Integrin	5	0	6	0
ICAM-1	5	5 (moderate to strong)	5	5 (moderate to strong)
VCAM-1	5	0	5	0
LECAM-2	5	0	6	0
E-cadherin	5	0	6	0
CD 44	6	6 (strong)	5	5 (strong)

cataract surgery, and collagen and fibronectin were identified.[22,23] Inhibition of the adhesion molecules may reduce such interactions between the IOL and LECs. The antibodies to some adhesion molecules reduced significantly anterior uveitis caused by intravitreous injection of IL-1 or endotoxin.[24] Our results suggest that migration of residual LECs onto the posterior capsule also might be hindered by the inhibition of integrin families or CD44 expressed by LECs.

Integrins are composed of two noncovalently associated transmembrane glycoprotein subunits, the α- and β-chains, both of which contribute to the binding to the matrix proteins. The binding of integrins to their ligands depends on the extracellular divalent cations (Ca^{2+} or Mg^{2+}) present in the α-chain. The β-chain of the integrins recognizes the RGD sequence (arginine-glycine-aspartic acid) present in ECM proteins. Therefore, chelation of Ca^{2+} by ethylenediaminetetraacetic acid may inhibit LEC migration by disrupting the adhesion to the lens capsule. The peptide RGD also may disrupt LEC migration by competitive binding to the integrins present on the surface of LEC, thereby inhibiting their binding to integrins on the lens capsule, as used experimentally for tumor metastasis inhibition.[25,26] We reported that sustained release of ethylenediaminetetraacetic acid significantly inhibited LEC migration onto the posterior capsule in rabbit eyes.[27] The results of sustained release of RGD will be reported elsewhere.

In conclusion, LECs of human cataracts express β_1 integrin, ICAM-1, and CD44 in situ and at 2 weeks of culture. These adhesion molecules may serve in the attachment of the LECs to the underlying lens capsule and be involved in the formation and disruption of cell-to-cell and cell-to-posterior capsule interactions when the LECs migrate after surgery onto the posterior capsule. Suppression or control of adhesion mole-

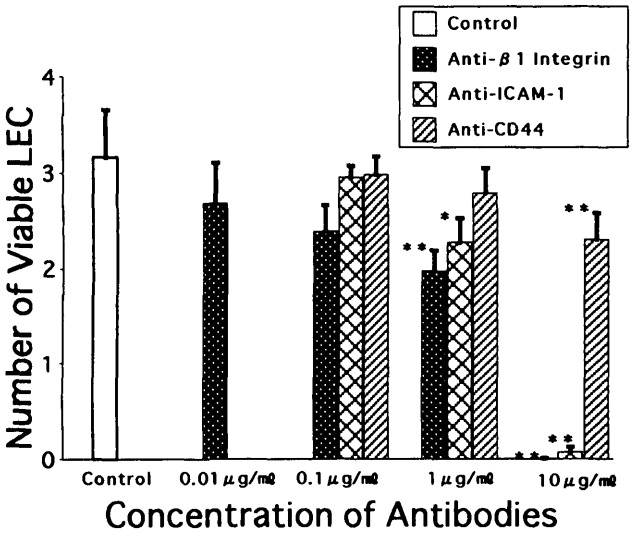

FIGURE 4. Functional assays of human cataract lens epithelial cell adhesion to the collagen-coated well. * and ** indicate significant differences at the level of $P < 0.05$ and $P < 0.01$, respectively, compared to the control.

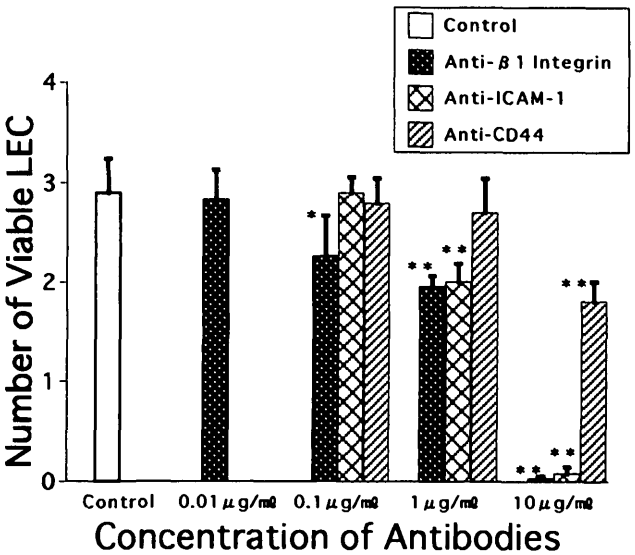

FIGURE 5. Functional assays of human cataract lens epithelial cell adhesion to the laminin-coated well.

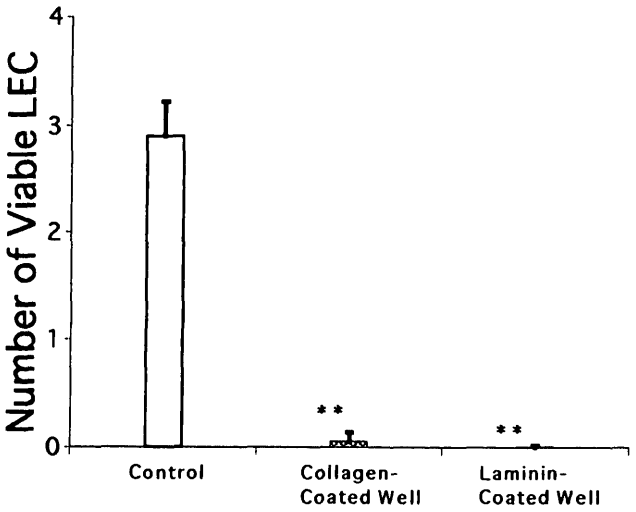

FIGURE 6. Functional assays of human cataract lens epithelial cell adhesion to the collagen- and laminin-coated plate when anti-β-1 integrin monoclonal antibody, anti-intercellular adhesion molecule-1 monoclonal antibody, and anti-CD44 monoclonal antibody were added to the incubation medium.

cules being produced by residual LECs may offer a new approach for inhibiting LEC proliferation or migration and, therefore, secondary cataract formation and inflammation. However, further investigations must be performed to delineate clearly the roles of adhesion molecules in LEC mitogenesis and migration.

Key Words

adhesion molecules, β_1 integrin, CD44, ICAM-1, lens epithelial cells, secondary cataract

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