

## SCIENTIFIC REPORT

# Detection of integrins in human cataract lens epithelial cells and two mammalian lens epithelial cell lines

S M McLean, M R K Mathew, J B Kelly, S B Murray, H G B Bennett, L A Webb, L Esakowitz, J S McLean

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**Aim:** To compare the incidence of various integrin subunits in human cataract anterior lens epithelial cells (A-LEC) and in two mammalian LEC lines.

**Methods:** Circular sections of anterior capsules with attached LEC were obtained during cataract surgery. Integrin subunits were immunolocalised in these anterior LEC and in a human and rabbit LEC line, using four monoclonal antibodies specific for subunits  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$ , and  $\beta$  subunit 2.

**Results:** All of these subunits were found in at least a proportion A-LEC samples as follows:  $\alpha 2$  71%,  $\alpha 3$  92%,  $\alpha 5$  62%, and  $\beta 2$  24%. The human LEC line was immunoreactive for  $\alpha 2$  and  $\alpha 3$  only. The rabbit lens epithelial cell line was immunoreactive for  $\alpha 5$  but there was no staining for  $\alpha 2$ ,  $\alpha 3$ , or  $\beta 2$ .

**Conclusion:** The A-LEC and mammalian LEC lines showed a similarity in their pattern of integrin expression. As these integrins are receptors for extracellular matrix (ECM) components, they are likely to be associated with the attachment and migration of LECs that precedes capsular opacification. Therefore these cell lines may be useful in the elucidation of mechanisms involved in the pathogenesis of capsular opacification.

Capsular opacification is a common complication of cataract surgery and occurs when residual lens epithelial cells (LEC) undergo fibrous metaplasia.<sup>1,2</sup> This involves LEC adhesion and migration and is dependent on integrin mediated cell-extracellular matrix (ECM) adhesion.<sup>3–5</sup>

Integrins are heterodimeric transmembrane glycoproteins, consisting of one  $\alpha$  and one  $\beta$  subunit, non-covalently linked. They bind to capsular ECM proteins, such as collagen type IV and laminin and also to fibronectin, present only in the embryonic lens capsule.<sup>6–9</sup> As integrins are cell surface adhesion molecules involved in epithelial cell attachment and are receptors for ECM components they may have a significant role in adhesion and migration of LEC across the lens capsule leading to anterior (ACO) or posterior capsule opacification (PCO).

Studies using LEC lines may elucidate mechanisms operating *in vivo* during the pathogenesis of both primary and secondary opacification. It is essential, however, to ensure that the properties of LEC lines are such that a valid comparison can be made between the behaviour of these cells *in vitro* and that of the cells which are responsible for capsular opacification *in vivo*.

We report here a comparison between expression of integrins on human cataract anterior lens epithelial cells (A-LEC) removed during cataract surgery and their expression by the immortalised human LEC line SRA 01/04 and rabbit LEC line N/N1003A. Understanding these differences

may help us develop better therapeutic strategies to prevent LEC migration and proliferation.

## MATERIALS AND METHODS

### Clinical specimens

Circular sections of anterior capsules with attached LEC were obtained from patients with age related cataract, (n = 95). All tenets of the declaration of Helsinki were followed.

### Immunohistochemical staining of anterior LECs

Capsules were dissected into four quadrants, each of which was stained immunohistochemically for one of four integrin subunits. Human anti-integrin antibodies used are listed in table 1. The site of primary antibody binding was detected using a commercially available peroxidase based detection system (Vectastain ABC kit, Vector Laboratories, Peterborough, UK).

In brief, anterior capsule quadrants were placed cell side up on slides coated with APES (3-aminopropyltriethoxysilane), fixed in acetone for 5 minutes, and allowed to air dry. Slides were then stored at  $-20^{\circ}\text{C}$  until used. Slides were washed with phosphate buffered saline (PBS) and endogenous peroxidase blocked with 3% aqueous hydrogen peroxide. After washing, primary antibody was applied for 60 minutes at  $37^{\circ}\text{C}$ , slides washed with PBS, and secondary antibody applied for 30 minutes at room temperature. Peroxidase activity was visualised by incubating with diaminobenzidine tetrachloride (DAB) for 10 minutes, at room temperature. Capsules were counterstained with haematoxylin. Primary antibody was omitted in negative control slides.

### Mammalian LEC lines

The immortalised human LEC line, SRA01/04, was cultured in Dulbecco's Modified Eagle Medium (DMEM), containing 20% (v/v) fetal bovine serum, penicillin (100 IU/ml), and streptomycin (50  $\mu\text{g/ml}$ ). The rabbit LEC line N/N1003A was cultured in Minimal Essential Medium Eagle (MEME) containing 10% (v/v) rabbit serum and gentamicin sulphate (50  $\mu\text{g ml}^{-1}$ ). Human LEC culture reagents were purchased from Invitrogen, Life Technologies, Paisley, UK. Rabbit LEC culture reagents were purchased from Sigma Aldrich UK. Cells were maintained at  $37^{\circ}\text{C}$  in a humidified, 5%  $\text{CO}_2$  atmosphere, medium changed twice weekly, and cells subcultured following trypsinisation.

### Immunocytochemical staining of LEC lines

LECs were grown on coverslips and subjected to indirect immunocytochemical staining using the EnVision System,

**Abbreviations:** ACO, anterior capsule opacification; A-LEC, anterior lens epithelial cells; DAB, diaminobenzidine tetrachloride; ECM, extracellular matrix; LEC, lens epithelial cells; PBS, phosphate buffered saline; PCO, posterior capsule opacification

**Table 1** Primary integrin antibodies used and possible dimmers

Antigen	Possible dimer(s)	Possible ligand(s)	Antibody	Antibody dilution
$\beta 2$	$\alpha 1\beta 2$	ICAM-1, 2; C3b1	CD18 (Novocastra)	1:25
$\alpha 2$	$\alpha 2\beta 1$	Collagen I, IV; laminin Collagen I, IV; laminin;	CD49b (Novocastra)	1:25
$\alpha 3$	$\alpha 3\beta 1$	fibronectin	CD49c (Novocastra)	1:25
$\alpha 5$	$\alpha 5\beta 1$	Fibronectin	CD49e (Novocastra)	1:25

HRP (Dako Ltd), in accordance with manufacturer's instructions. Incubation times were identical to those used for anterior capsules. Antibodies are detailed in table 1. Negative controls were incubated in PBS instead of the primary antibody.

## RESULTS

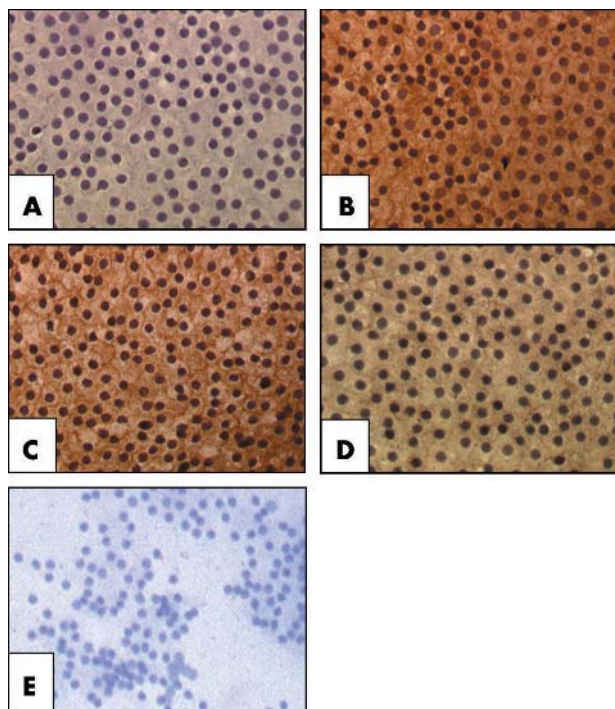
### Anterior capsules

A-LEC obtained from cataractous lenses were analysed for  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 5$ , and  $\beta 2$  integrin subunit immunoreactivity. There was positive staining for  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$  and, to a lesser extent,  $\beta 2$  (fig 1).

Anterior capsules were taken from 95 patients, but in a few cases, capsule quadrants had no attached LEC, hence the variability in sample size. A significantly higher proportion of A-LEC showed positive staining for  $\alpha 3$  (95% CI =  $p < 0.001$ ) compared to  $\alpha 2$  (95% CI = 1.64,  $p < 0.001$ ),  $\alpha 5$  (95% CI = 2.09,  $p < 0.001$ ), and  $\beta 2$  (95% CI = 2.83,  $p < 0.001$ ) (table 2). No positive staining was observed in the negative controls.

### Human lens epithelial cells

The SRA01/04 cells were immunoreactive for  $\alpha 2$  and  $\alpha 3$ . No staining was obtained for  $\alpha 5$  and  $\beta 2$ . As before, no staining was observed in the negative controls (fig 2).



**Figure 1** Staining of anterior lens epithelial cells for integrin subunits. (A)  $\beta 2$ , (B)  $\alpha 2$ , (C)  $\alpha 3$ , (D)  $\alpha 5$ , (E) negative control. Cell nuclei are stained blue and LECs positive for integrin subunits show brown staining. Original magnification  $\times 400$ .

### Rabbit lens epithelial cells

The N/N1003A cell line stained positively for only  $\alpha 5$ , but there was no staining for  $\alpha 2$ ,  $\alpha 3$ , or  $\beta 2$  (fig 2).

## DISCUSSION

Several studies have shown that residual A-LEC undergo proliferation, migration, and epithelial mesenchymal transformation to myofibroblast-like cells leading to capsular opacification and visual compromise.<sup>3-5</sup>

The lens capsule components, collagen type IV, and laminin have RGD recognition sequences for integrins on adjacent LECs.<sup>7, 8, 10-13</sup>

Our results show that integrins of two different subfamilies ( $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 5$ , and  $\beta 2$ ) are present in A-LEC, whereas the human SRA01/04 cell line expresses  $\alpha 2$  and  $\alpha 3$  and the N/N1003A cell line expresses only  $\alpha 5$ . The  $\alpha 3$  subunit was found in 92% of capsules studied in comparison with  $\alpha 2$ , (71%),  $\alpha 5$  (62%) and  $\beta 2$  (24%). The  $\alpha 3\beta 1$  heterodimer is considered responsible for the adherence of cells to extracellular matrix components such as collagen type IV and laminin whereas the  $\beta 2$  subunit is associated with cell to cell adhesion.<sup>14, 15</sup>

Zhang and colleagues<sup>7</sup> found  $\beta 1$ ,  $\beta 2$ ,  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$  integrins in 70%, 65%, 75%, 70%, and 80% of human cataract A-LEC, respectively. In the present larger study the higher prevalence of  $\alpha 3$  compared to  $\alpha 2$ ,  $\alpha 5$ , and  $\beta 2$  subunits may reflect differing profiles of patient samples. Nishi and colleagues<sup>8</sup> described the detection of  $\beta 1$  integrins (and also ICAM-1 and CD 44) in circular sections of anterior capsule taken during cataract surgery, both immediately ex vivo and after 2 weeks of culture, but made no attempt to identify  $\alpha$  subunits.

The differences in expression of integrins by A-LEC and SRA01/04 cells may reflect the paediatric origin of the cell line, whereas A-LEC were from patients with maturity onset cataract.

The expression of  $\alpha 5$  and  $\beta 2$  in anterior capsules may indicate induction of these subtypes in maturity onset cataracts.  $\alpha 5$  is known to bind fibronectin, and has been detected in myofibroblast-like LECs in patients with anterior subcapsular cataract<sup>13</sup> and induction may be related to fibronectin deposition. The absence of expression of these units in SRA01/04 cells may be a result of immortalisation, a reflection of the paediatric nature of the primary cells or associated with in vitro culture conditions. Expression of  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$  integrin subunits by SRA01/04 cells has been previously reported.<sup>10</sup> Note that in our hands SRA01/04 cells were not immunoreactive for  $\alpha 5$  integrin (fibronectin receptor), not surprising in a cell line not derived from embryonic tissue. The  $\alpha 5$  integrin subunit was found in fewer anterior capsule samples than  $\alpha 2$  and  $\alpha 3$ , but was present in the N/N1003A cell line, an appropriate finding in a cell line derived from neonatal rabbits.

Integrin expression has been investigated in LEC lines from several species. Lim and colleagues<sup>11</sup> demonstrated the presence of  $\alpha 3$ ,  $\alpha 5$ ,  $\alpha 6$ , and  $\alpha v$  integrin in a human LEC line (HLE-B3) and the murine LEC line,  $\alpha TN4$ . Expression of  $\alpha 5$

**Table 2** Individual expression of integrins in cataract A-LEC

Antigen	Antibody	No of quadrants with attached LECs		Percentage
		Positive	Negative	
$\alpha 2$	CD49b	89	63	71
$\alpha 3$	CD49c	91	83	92
$\alpha 5$	CD49e	94	58	62
$\beta 2$	CD18	93	22	24

integrin was downregulated by TGF  $\beta 1$  whereas  $\alpha 3$ ,  $\alpha 6$ , and  $\alpha v$  expression was unaffected. To our knowledge integrins have not been studied in the N/N1003A cell line. Our results highlight the variation in the pattern of integrin expression and possible subsequent adhesion and migration by two LEC lines.

Cell adhesion molecules, especially integrins, are signal transducers within the cell, as well as receptors for extracellular matrix. Integrin ligation to matrix components causes a conformational change in the cytoplasmic tail of integrin, which allows it to bind to the actin cytoskeleton and

signal transduction molecules.<sup>16, 17</sup> This allows integrins to act as bidirectional signal transducers.<sup>18</sup>

The  $\alpha 2$  and  $\alpha 3$  integrin subunits bind collagen type IV and laminin respectively, and as both of these extracellular matrix molecules are components of the lens capsule, the presence of these integrin subunits is not unexpected. SRA01/04 cells were found to express the  $\alpha 2$  and  $\alpha 3$  integrin subunits, confirming the suitability of these cells for further adhesion and migration studies.

In our study, each integrin subtype was not found in ALEC of every capsule tested. This may reflect individual characteristics of each patient. Similarly, N/N1003A and SRA01/04 cell lines had differing patterns of integrin subunit expression. This may be a consequence of the different primary cells from which these cell lines were derived, or associated with differing in vitro culture conditions.

Although there have been several investigations on the expression of integrins in LEC lines, there has been no direct comparison with human cataract A-LEC.

It is clear that both N/N1003A and SRA01/04 cell lines have integrin expression profiles very close to those found in LEC from cataract patients. As integrins play an important part in the cascade of events leading to capsule opacification, these cell lines provide an ideal tool to investigate the pathogenesis of this process.

This would have clinical implications for the development of new therapeutic approaches to inhibit capsule opacification caused specifically by residual LEC.

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## Authors' affiliations

S M McLean, J B Kelly, J S McLean, Department of Biological Sciences, University of Paisley, Paisley PA1 2BE, UK

M R K Mathew, Department of Ophthalmology and Visual Sciences, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK

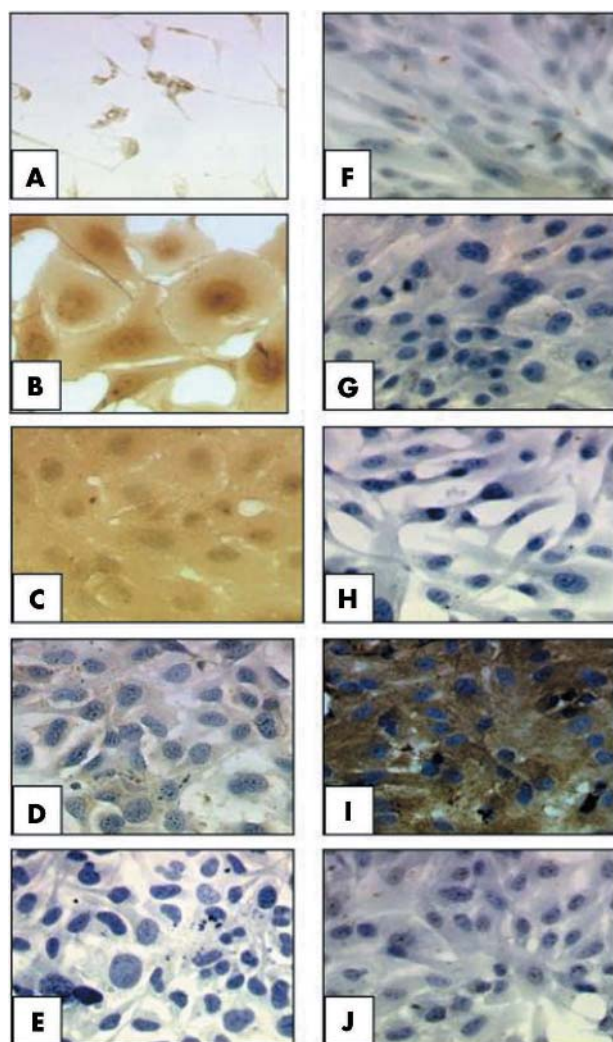
H G B Bennett, S B Murray, L A Webb, L Esakowitz, Department of Ophthalmology, Argyll and Clyde Acute Hospitals NHS Trust, Paisley PA2 9PN, UK

Correspondence to: Dr S M McLean, Department of Biological Sciences, University of Paisley, Paisley PA1 2BE, UK; [stella.mclean@paisley.ac.uk](mailto:stella.mclean@paisley.ac.uk)

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**Figure 2** Staining of lens epithelial cell lines for integrin subunits. SRA01/04 cell line (A)  $\beta 2$ , (B)  $\alpha 2$ , (C)  $\alpha 3$ , (D)  $\alpha 5$ , (E) negative control. N/N1003A cell line (F)  $\beta 2$ , (G)  $\alpha 2$ , (H)  $\alpha 3$ , (I)  $\alpha 5$ , (J) negative control. Cell nuclei are stained blue and LECs positive for integrin subunits show brown staining. Original magnification  $\times 400$ .

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