

Abnormal expression of integrin $\alpha 6 \beta 4$ in cervical intraepithelial neoplasia

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Summary We have used subunit-specific monoclonal antibodies (MAbs) and immunohistochemistry to examine the distribution of integrin $\alpha 6 \beta 4$ in normal ectocervical epithelium and various grades of cervical intraepithelial neoplasia (CIN). Antibodies were first characterised by immunoprecipitation from two surface-labelled tumour cell lines. Monoclonal antibody G71 was found to precipitate integrin $\beta 4$ from BeWo but not T47D cells, while other anti- $\beta 4$ antibodies precipitated $\beta 4$ from both cell lines. Both G71 and an antiserum to the C-terminal peptide of $\beta 4$ precipitated free $\beta 4$ from surface-iodinated BeWo cells. Neither antibody recognised truncated $\beta 4$ chains observed at approximately 160 kDa. These data suggest that different isoforms of $\beta 4$ are expressed in different tumour cell lines, and that there may be a pool of $\beta 4$ at the cell surface that is not complexed to $\alpha 6$. In normal cervix, both the $\alpha 6$ and $\beta 4$ subunits occur at the basal surface of the basal cell layer. In CIN, the distribution is markedly altered, with strong expression of $\alpha 6$ and $\beta 4$ in the upper cell layers of the ectocervical epithelium. All 40 cases of CIN that were studied exhibited this alteration. Furthermore, the extent of extrabasal staining appeared to correspond with the grade of CIN. The form of integrin $\beta 4$ recognised by antibody G71 also appears in the upper cell layers in CIN, but it shows a more restricted distribution than the normal isoform.

Keywords: integrin $\alpha 6 \beta 4$; cervix; cervical intraepithelial neoplasia

Integrins are a family of heterodimeric ($\alpha\beta$) cell-surface receptors involved in cell–matrix and cell–cell interactions (Hynes, 1992). Integrin $\alpha 6 \beta 4$ has been shown to be expressed by many epithelial cells, usually at the basal cell surface at the site of adhesion to the basement membrane (Sonnenberg and Linders, 1990). Direct evidence for the involvement of $\alpha 6 \beta 4$ in cell–basement membrane interaction has come from tissues containing hemidesmosomes, in which it has been demonstrated that $\alpha 6 \beta 4$ is specifically localised within these anchorage structures (Stepp *et al.*, 1990; Sonnenberg *et al.*, 1991; Behzad *et al.*, 1995). There is evidence that laminin functions as a ligand for $\alpha 6 \beta 4$ (De Luca *et al.*, 1990; Lotz *et al.*, 1990; Lee *et al.*, 1992; Niessen *et al.*, 1994; Aplin and Church, 1995). Mutations of the $\beta 4$ subunit have been observed in junctional epidermolysis bullosa, where there is loss of dermal–epidermal adhesion (Phillips *et al.*, 1994; Vidal *et al.*, 1995).

Cell surface molecules that mediate cell–cell or cell–matrix adhesive interactions may be altered qualitatively or quantitatively in carcinoma. Such alterations during neoplastic transformation (Giancotti and Ruoslahti, 1990; Symington, 1990; Plantefaber and Hynes, 1989; Risinger *et al.*, 1994; Tidman *et al.*, 1990) are coupled with the disruption of basement membrane integrity and may occur as a prerequisite of invasion into the underlying stroma (Liotta *et al.*, 1991; Frixen *et al.*, 1991). Some of these changes are likely to be detectable in preinvasive phases. Knowledge of the altered adhesive properties of transformed cells also offers increased insight into the natural history of the disease (Liotta *et al.*, 1991; Giancotti and Ruoslahti, 1990; Symington, 1990; Plantefaber and Hynes, 1989).

Invasive cervical cancer is preceded by a variable period of intraepithelial neoplasia (CIN) thus providing an opportunity to study neoplastic transformation in the preinvasive phase. Here we describe the results of our investigation into the behaviour of integrin $\alpha 6 \beta 4$ in CIN, where altered adhesive properties are likely to be important in the development of invasive cells.

Materials and methods

Cervical biopsies

Cervical biopsies ($n=40$) were selected from areas showing abnormalities according to the standard colposcopic criteria of the presence of acetowhite, iodine-negative lesions and vascular abnormalities. Appropriate local ethical permission was obtained. The biopsies were first washed in phosphate-buffered saline (PBS), oriented correctly, then snap frozen onto cryostat stubs in liquid nitrogen, using Optimal Cutting Temperature Compound (OCT; Orme Scientific). They were sectioned at right angles to the epithelial/stromal junction at 7 μm onto precleaned microscope slides (Taab) on a Reichart Jung E cryostat and stored at -80°C until required. Normal cervical tissues were obtained at hysterectomy from women reported as having recent normal cytology. Initial histopathological assessment indicating normality or the presence of CIN and its grade was made on serial sections. The diagnosis was later confirmed independently by another histopathologist who screened the entire series.

Antibodies

Mouse monoclonal antibody (MAb) 5B5 to integrin $\beta 4$ was raised against amnion epithelial cells and had been previously characterised by immunoprecipitation from $\beta 4$ -positive and -negative cell lines (Sonnenberg *et al.*, 1991; Aplin *et al.*, 1992). Mouse MAb G71 to integrin $\beta 4$ was raised against epithelial cells obtained from endometrial tissue (Aplin and Seif, 1985; Aplin *et al.*, 1992). Rat MAb GoH3 to integrin $\alpha 6$ (Sonnenberg and Linders, 1990) was a generous gift from Dr Arnoud Sonnenberg, Amsterdam. Rat MAb 439-9B to integrin $\beta 4$ (Sonnenberg *et al.*, 1991) was a kind gift from Dr Steven Kennel, Oak Ridge, Tennessee. Monoclonal antibodies were used as appropriately diluted hybridoma supernatants.

Rabbit antiserum to integrin $\beta 4$ was raised to the synthetic peptide TLSTHMDQQFFQTC based on the cytoplasmic carboxy terminal sequence of the molecule. This was conjugated to rabbit serum albumin and used in repeated subcutaneous injections. The serum was characterised by ELISA on the peptide conjugate and Western blotting on the intact subunit. It was then immunoaffinity purified for use in immunoprecipitation.

Immunoprecipitation analysis

Human choriocarcinoma (BeWo) and breast carcinoma (T47D) cells have been shown to express integrin subunits on their cell surface and were used as controls. They were grown in a 1:1 mixture of Ham's F12 and Dulbecco's modified Eagle medium with 10% fetal calf serum (FCS), Hepes, 2 mM L-glutamine, gentamycin and streptomycin. BeWo human choriocarcinoma cells were grown to confluency in Dulbecco's modified Eagle medium supplemented with 10% FCS, glutamine and antibiotics. Cells were surface labelled with ^{125}I (1 mCi per 10^{-7} cells) using lactoperoxidase/hydrogen peroxide (Aplin *et al.*, 1992) and lysed with 2% Triton X100, 5 mg ml $^{-1}$ bovine serum albumin (BSA), 10 mg ml $^{-1}$ leupeptin and 2 mM phenylmethylsulphonyl fluoride (PMSF) in PBS ABC (1 ml 10^{-7} cells). The resulting lysate was precleared with protein A-Sepharose (Sigma), preloaded with a similar Triton extract made using non-radioactive cells. The supernatant was divided into aliquots for immunoprecipitation; to 25 ml of antibody (GoH3, 5B5 and G71), 150 ml of iodinated cell extract was added in a total volume of 200 ml in PBS ABC and the reaction mixture incubated on ice for 1 h with periodic mixing. Immune complexes were collected with protein A-Sepharose pre-blocked with cold cell extract and preloaded with anti-mouse immunoglobulin (Dako) at a concentration of 1 mg IgG per ml of packed beads. This bead preparation, which is loaded with anti-mouse IgG to only a fraction of its total binding capacity, was used for both mouse and rabbit primary antibodies; the latter, used as a polyclonal preparation, binds avidly to unoccupied protein A. The beads were washed six times with 1% Triton X100 in PBS ABC followed by a final wash in PBS ABC and boiled in gel loading buffer for 10 min. Immunoprecipitates were analysed by SDS-PAGE on 5% gels followed by autoradiography.

Immunohistochemistry

Stored, frozen sections of cervical tissue were brought to room temperature and fixed in cold acetone for 10 min, followed by washing in PBS. Endogenous peroxidase activity was blocked for 60 min at 37°C (Andrew and Jasani, 1987). Sections were then washed in PBS. All antibody incubations were carried out for 60 min at room temperature, with three 5 min washes in PBS between stages. Primary antibody was used at 1/25 (v/v) in PBS. Secondary antibody was biotinylated rabbit anti-mouse (Dako) used at 1/300 (v/v). A drop of avidin in complex with biotinylated peroxidase (ABCComplex/HRP; Dako) was then placed on the sections and left for 30 min, rinsed off and washed as before. Bound antibody was visualised by incubation in 3,3'-diaminobenzidine (DAB) for 5–10 min (200 mg of DAB dissolved in 400 ml PBS, filtered, with the addition of 60 ml hydrogen peroxide). After rinsing in running tap water, sections were counterstained in Harris's haematoxylin (Sigma), dehydrated and mounted in Hystomount (Taab). Appropriate controls were included in each run.

Sections were graded for the extent of extrabasal staining. H & E-stained consecutive sections were analysed and graded independently for the extent of CIN by a histopathologist.

Results

Characterisation of antibodies by immunoprecipitation

Two carcinoma cell lines which express integrin $\alpha 6 \beta 4$ – BeWo (Aplin *et al.*, 1992) and T47D (Sonnenberg and Linders, 1990) – were selected for the characterisation of subunit-specific monoclonal antibodies to be used in the study. Cell surface iodination was carried out before immunoprecipitation from detergent extracts under conditions expected to preserve the association of integrins into heterodimeric complexes. The anti-integrin $\alpha 6$ monoclonal antibody GoH3 precipitated from T47D cells the $\alpha 6$ subunit (120 kDa under

reducing conditions) along with the 200 kDa $\beta 4$ chain with which it is associated (Figure 1, lane 1). In addition, a pair of closely spaced bands was visible at approximately 160 kDa; these are probably truncated forms of integrin $\beta 4$ also found in complex with $\alpha 6$ (Falcioni *et al.*, 1988; Kennel *et al.*, 1989;

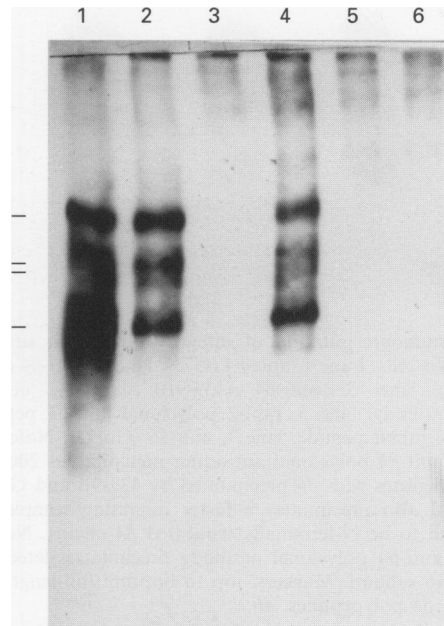


Figure 1 Immunoprecipitation of integrin $\alpha 6 \beta 4$ from surface-iodinated T47D cells. Lane 1, anti- $\alpha 6$ (GoH3); lane 2, anti- $\beta 4$ (439-9B); lane 3, control monoclonal antibody; lane 4, anti- $\beta 4$ (5B5); lane 5, control monoclonal antibody; lane 6, G71. Note that GoH3, 439-9B and 5B5 all precipitate bands migrating in the $\alpha 6$ and $\beta 4$ positions, as well as truncated forms of $\beta 4$. No reactivity is detected in the G71 or control lanes. Markers, top to bottom: full-length $\beta 4$, two truncated $\beta 4$ polypeptides, $\alpha 6$.

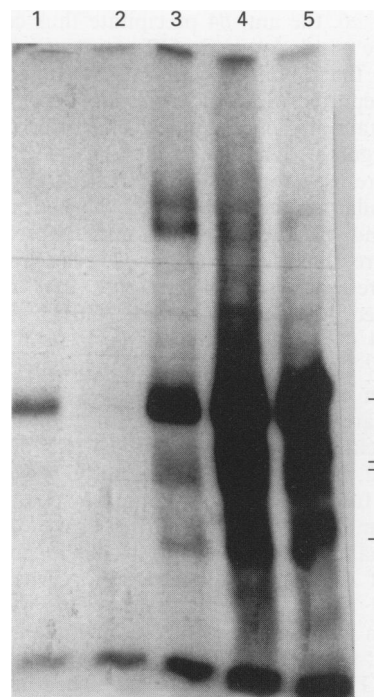


Figure 2 Immunoprecipitation of integrin $\alpha 6 \beta 4$ from surface-iodinated BeWo cells. Lane 1, anti- $\beta 4$ (G71); lane 2, control monoclonal antibody; lane 3, anti- $\beta 4$ (5B5); lane 4, anti- $\beta 4$ (439-9B); lane 5, anti- $\alpha 6$ (GoH3). Note that G71 precipitates a band comigrating with the $\beta 4$ chain. 5B5, 439-9B and GoH3 all precipitate $\beta 4$, truncated forms of $\beta 4$ and $\alpha 6$. Markers, top to bottom: full length $\beta 4$, two truncated $\beta 4$ polypeptides, $\alpha 6$.

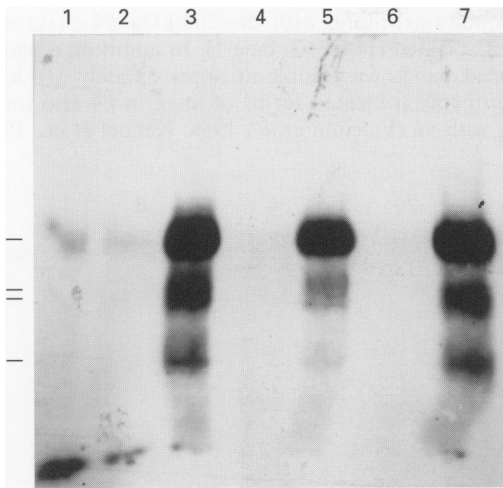


Figure 3 Immunoprecipitation of integrin $\alpha 6 \beta 4$ from surface-iodinated BeWo cells. Lane 1, anti- $\beta 4$ (G71); lane 2, anti- $\beta 4$ (5B5) (underloaded); lane 3, anti- $\beta 4$ (439-9B); lane 4, control monoclonal antibody; lane 5, rabbit polyclonal anti- $\beta 4$ peptide; lane 6, control rabbit peptide; lane 7, anti- $\alpha 6$ (GoH3). Note that G71 and the anti- $\beta 4$ polyclonal antiserum precipitate a 200 kDa band that comigrates with $\beta 4$ precipitated by 439-9B and GoH3. The polyclonal also precipitates a faster migrating complex of bands, deduced to be N-terminally truncated $\beta 4$ chains. Neither G71 nor the anti- $\beta 4$ polyclonal antibody precipitates detectable quantities of $\alpha 6$ subunit. Markers, top to bottom: full-length $\beta 4$, two truncated $\beta 4$ polypeptides, $\alpha 6$.

Van Waes *et al.*, 1991; Aplin *et al.*, 1992; Giancotti *et al.*, 1992; Potts *et al.*, 1994).

Antibody 439-9B to integrin $\beta 4$ gave a similar pattern (Figure 1, lane 2), though somewhat lower $\alpha 6$ subunit signal intensity was detected. This suggested that, as previously noted (Sonnenberg and Linders, 1990), a fraction of the pool of integrin $\beta 4$ at the surface of T47D cells may be unassociated with $\alpha 6$, the anti- $\beta 4$ precipitate thus containing a lower abundance of $\alpha 6$. Antibody 5B5 to integrin $\beta 4$ gave a result identical to that obtained with 439-9B (Figure 1, lane 4) as previously reported (Sonnenberg *et al.*, 1991). We were unable to precipitate any polypeptide from T47D cells with antibody G71 (Figure 1, lane 6).

BeWo cells were used to investigate further the properties of G71. Very similar results were obtained when antibodies GoH3, 439-9B and 5B5 were used in immunoprecipitation from BeWo (Figure 2) and T47D (Figure 1) cells. Like T47D, BeWo cells expressed an $\alpha 6 \beta 4$ complex that could be immunoprecipitated with either anti- $\alpha 6$ (GoH3: Figure 2, lane 5) or anti- $\beta 4$ (439-9B: Figure 2, lane 4; 5B5: Figure 2, lane 3) antibodies. Truncated $\beta 4$ chains were again present at about 160 kDa in each case. Antibody G71 precipitated a band that comigrated precisely with the full length $\beta 4$ chain (200 kDa), but neither $\alpha 6$ nor truncated $\beta 4$ was detected in this case (Figure 2, lane 1). To confirm that G71 was indeed recognising the full length $\beta 4$ chain, we compared its behaviour in immunoprecipitation from BeWo cells with that of a polyclonal antibody raised to an oligopeptide based on the published C-terminal sequence (Hogervorst *et al.*, 1990; Suzuki and Naitoh, 1990; Tamura *et al.*, 1990). The anti-peptide serum immunoprecipitated the 200 kDa $\beta 4$ chain (Figure 3, lane 5), which comigrated precisely with the chain precipitated by G71 (Figure 3, lane 1). The anti-peptide serum also precipitated a doublet of bands at approximately 160 kDa (Figure 3, lane 5). These must be assumed to be N-terminally truncated forms of $\beta 4$, also reported by Giancotti *et al.* (1992). The bands are substantially weaker than those seen in this molecular weight range when 439-9B (Figure 3, lane 3) or GoH3 (Figure 3, lane 7) were used, suggesting that C-terminally truncated forms of $\beta 4$ are also present (Falcioni *et al.*, 1988; Kennel *et al.*, 1989; Van Waes *et al.*, 1991;

Giancotti *et al.*, 1992); these would not be recognised by the peptide antiserum. Neither G71 nor the peptide antiserum precipitated detectable quantities of the integrin $\alpha 6$ chain. This suggests that free $\beta 4$ is present at the BeWo cell surface, and that both these antibodies preferentially recognise it.

The distribution of integrin $\alpha 6 \beta 4$ in cervical tissue

Monoclonal antibodies GoH3, G71 and 5B5 were used to examine the distribution of integrin $\alpha 6 \beta 4$ in normal and neoplastic cervix. The expression of the $\beta 4$ integrin subunit in normal stratified squamous epithelium was monitored by antibody 5B5. Expression was strongest at the basal aspect of the basal cells (Figure 4a and b), with an accompanying weaker pericellular and diffuse staining pattern in the basal and parabasal layers (Figure 4b). The $\alpha 6$ subunit gave a similar distribution (Figure 4c) as monitored by staining with GoH3.

In cases of CIN (Figure 4d and e), staining with 5B5 and GoH3 was seen on the surface of neoplastic cells which displayed nuclear abnormalities, including an increased nucleocytoplasmic ratio, nuclear hyperchromatism and the presence of abnormal mitotic figures. In CIN I, in which abnormal cells occupy the lower one-third of the epithelium, staining with 5B5 extended beyond the basal region with strong pericellular staining of the abnormal cells. In CIN II, as the abnormal cells extend further through the epithelium to occupy up to two-thirds of its thickness, staining with 5B5 and GoH3 followed suit. In CIN III, pericellular staining was observed throughout the full thickness of epithelium though there was some variation in its intensity (Figure 4d and e). Altogether 40 cases of CIN were studied, and all of them conformed to this pattern of staining.

Antibody G71 showed similar behaviour, with predominantly basal staining in the normal ectocervical epithelium (Figure 5a). This antibody did not stain the lateral plasma membranes or cytoplasmic regions of basal or parabasal cells. The epitope was also present in the walls of small blood vessels in the upper dermis (Figure 5a and b). G71 staining was increasingly visible in the upper layers of the epithelium with increasing grade of CIN (Figure 5b). Although G71-positive cells could be observed throughout the epithelium in CIN III, extrabasal staining was more heterogeneous and less extensive than observed with 5B5 or GoH3, with G71-negative cells always present in the lesion.

Discussion

Cervical cancer is one of the major health care issues affecting women in the UK despite the proven success of screening programmes for the preinvasive lesions (CIN) (Anderson *et al.*, 1988). The mechanisms that are involved in the progression of intraepithelial neoplasia from *in situ* to invasive phenotype remain poorly understood, but include changes in cell adhesion, motility and proteolytic activity leading to altered stability of the basement membrane associated with penetration into the sublaminal matrix (Liotta *et al.*, 1991).

All available evidence indicates that the integrin $\beta 4$ subunit forms heterodimeric complexes uniquely with integrin $\alpha 6$ (Carter *et al.*, 1990; De Luca *et al.*, 1990; Lotz *et al.*, 1990; Sonnenberg and Linders, 1990; Sonnenberg *et al.*, 1991; Lee *et al.*, 1992). However, in addition to its heterodimeric form, integrin $\beta 4$ has been suggested to exist at the cell surface in a form uncomplexed to a chain (Sonnenberg and Linders, 1990). Our data demonstrating that anti- $\beta 4$ antibodies 5B5 and 439-9B precipitate relatively more labelled $\beta 4$ and less $\alpha 6$ than does antibody GoH3 to the $\alpha 6$ chain can be adduced in support of this suggestion, as can the data of Hodivala *et al.* (1994) in cultured keratinocytes.

In normal cervix we have detected the $\alpha 6$ and $\beta 4$ subunits strongly at the basal cell surface, but also more weakly in the cytoplasm and lateral plasma membrane domain of both

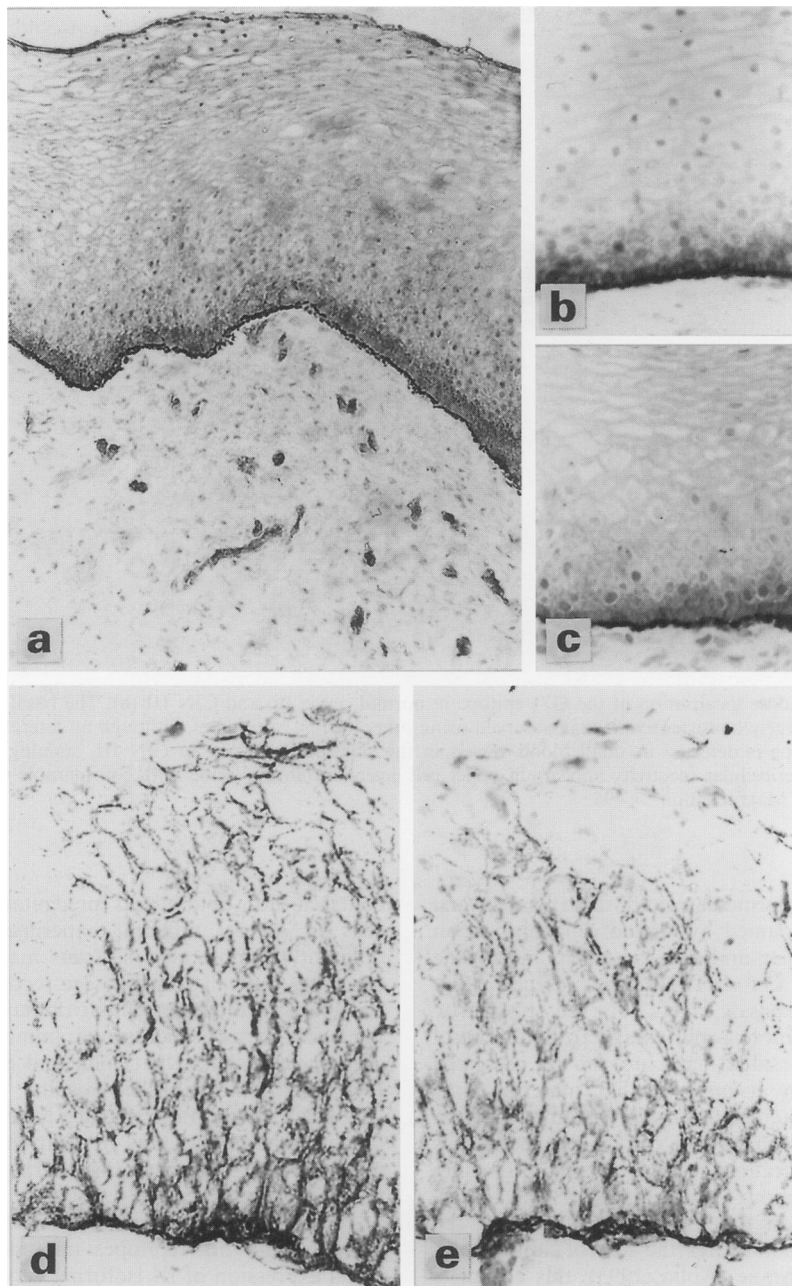


Figure 4 Comparison of the distribution of integrin subunits $\beta4$ (antibody 5B5) and $\alpha6$ (antibody GoH3) in normal cervix and CIN III by immunoperoxidase histochemistry. (a, b) 5B5, normal cervix; (c) GoH3, normal cervix; (d) 5B5, CIN III; (e) GoH3, CIN III. Note the strongly basal localisation of both subunits in normal tissue. In CIN III, both subunits show a pericellular pattern of staining extending throughout the epithelium. Magnifications: a, $\times 80$; b, c, $\times 160$; d, e, $\times 475$.

basal and parabasal cells. This distribution was also reported by Carico *et al.* (1993) but it differs slightly from other reports in which a basal-only pattern has been described (Sonnenberg and Linders, 1990; Lee *et al.*, 1992; Hughes *et al.*, 1994). The distinction is probably a result of the combination of a high-affinity antibody with a highly sensitive staining protocol, allowing the detection of smaller quantities of antigen. All authors agree that the major location of the $\alpha6\beta4$ complex is at the basal cell surface. In cornea (Stepp *et al.*, 1990), skin (Sonnenberg *et al.*, 1991) and amnion (Behzad *et al.*, 1995) it is known to be concentrated specifically in hemidesmosomal junctions, where it is assumed to have a role in anchoring the cell surface to the extracellular matrix. Other normal epithelial cells that lack hemidesmosomes also express $\alpha6\beta4$ basally (Sonnenberg and Linders, 1990; Aplin, 1993).

The observation of a disrupted pattern of expression of both $\alpha6$ and $\beta4$ (and by inference, $\alpha6\beta4$) in CIN confirms the

results reported by Carico *et al.* (1993) but we have used a significantly larger study group (40 vs 13 respectively). Hughes *et al.* (1994) reported similar findings in the context of a larger panel of integrins. Our data are also consistent with previous work indicating elevated levels of the $\beta4$ subunit in murine carcinoma (Falcioni *et al.*, 1988; Kennel *et al.*, 1989; Van Waes *et al.*, 1991), and with our previous observation of increased expression in extrabasal locations in squamous cell carcinoma of the skin (Tidman *et al.*, 1990). In contrast, Hodivala *et al.* (1994) reported a reduced level of integrin $\alpha6\beta4$ expression in HPV16/Ha-*ras*-transformed keratinocytes and in a small group of HPV-positive CIN lesions. In this context it will be of interest to correlate further the behaviour of integrin $\alpha6\beta4$ with HPV status in CIN.

The onset of proliferative intraepithelial change implies altered tissue kinetics with more rapid escape of neoplastic cells from the basal layers of the tissue as well as loss of

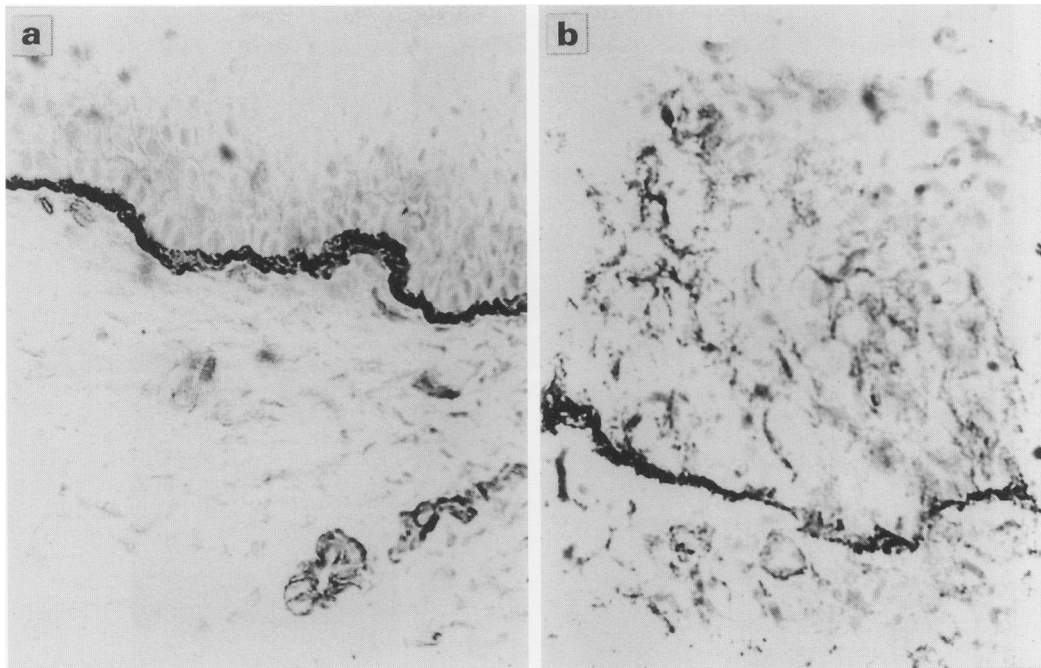


Figure 5 Immunoperoxidase localisation of the G71 epitope in normal cervix (a) and CIN III (b). The basal localisation observed in the normal tissue is largely consistent with that observed using other anti- $\beta 4$ antibodies, although no lateral staining is observed. Note also that the epitope is detected in small blood vessels in the superficial dermis. In CIN III, staining in the basal layer is somewhat weaker, but pericellular reactivity appears in upper cell layers within the lesion with considerable variation in reactivity between individual cells. Magnification: $\times 495$.

differentiated cells from intermediate and superficial layers. This is likely to be accompanied by altered cell polarisation and architecture, intercellular and cell–basement membrane adhesive interactions (Liotta *et al.*, 1991). The presence of $\beta 4$ -containing cells in extrabasal layers of the neoplastic epithelium could imply a more rapid escape from the basal layer of cells bearing the residual hallmarks of the basal cell phenotype, or alternatively it may be that integrin $\beta 4$ is capable of a function in intercellular organisation other than adhesion to basement membrane. Loss of hemidesmosomes is a prerequisite of cell migration, either in wound healing (Kurpakus, 1991) or tumour invasion (McNutt, 1976). During trophoblast invasion loss of $\alpha 6 \beta 4$ occurs from the migrating cell population soon after the loss of adhesion to the villous basement membrane (Aplin, 1993); this contrasts with the persistence of $\alpha 6 \beta 4$ in cervical neoplastic cells *in situ* as well as in invasive foci (Carico *et al.*, 1993; Hughes *et al.*, 1994).

The properties of monoclonal antibodies 5B5 and 439-9B show clearly that they recognise the extracellular domain of integrin $\beta 4$ (Sonnenberg *et al.*, 1991) and immunoprecipitate $\alpha 6 \beta 4$ complexes. In addition to the full length polypeptide, these antibodies capture truncated forms of the $\beta 4$ chain present in both cell lines. The properties of these isoforms are similar to those described previously for C-terminally truncated $\beta 4$ chains (Falcioni *et al.*, 1988; Kennel *et al.*, 1989; Van Waes *et al.*, 1991; Giancotti *et al.*, 1992). These can also be captured using antibody to $\alpha 6$, confirming their ability to complex stably with a $\beta 4$ chain. We cannot exclude that some proteolysis of $\beta 4$ occurred during our experiments since calcium ions were present, and Giancotti *et al.* (1992) have shown that the cytoplasmic domain of the subunit is sensitive to a calcium-dependent protease, calpain. Their evidence suggests that truncated forms of $\beta 4$ are present along with the full length chain *in vivo*. The immunoprecipitation analysis reported here, in which we have demonstrated that G71 recognises a 200 kDa chain in BeWo cells with precisely the same electrophoretic properties as one recognised by a polyclonal antibody to the C-terminus of human integrin $\beta 4$, confirms previous evidence (Aplin *et al.*, 1992) that G71 recognises the integrin $\beta 4$ subunit. In

contrast, no subunit was precipitated by G71 from T47D cells in several different experiments. This suggests that different $\beta 4$ molecular isoforms may be present in different cell types, not all of which are recognised by G71. The G71 epitope is in the extracellular domain of $\beta 4$ as monitored by light and electron microscopic immunolocalisation in normal amnion epithelial cells (Aplin and Seif, 1985; JD Aplin and DR Garrod, unpublished results). G71 recognises the full length $\beta 4$ subunit in preference to shorter variants suggesting that its binding site may be near the amino terminus of the molecule and therefore lost during N-terminal proteolytic truncation.

It was therefore of interest to compare the distribution of the G71 and 5B5 epitopes immunohistochemically. Differential expression of $\beta 4$ isoforms may reflect the heterogeneity of neoplastic cell behaviour and could be relevant to pathophysiology of different phenotypes. The greater heterogeneity of binding of G71 to neoplastic cells suggests that a varying degree of proteolytic or other modification may occur after escape from the basement membrane.

We have shown that $\alpha 6 \beta 4$ integrin displays an abnormal distribution with remarkable consistency and in a fashion that reflects the extent and grade of CIN. The appearance of $\alpha 6 \beta 4$ in association with neoplastic cells suggests the possibility that it may also occur in detectable quantities in cervical smears. Our data support the view that the analysis of molecular changes occurring in preinvasive conditions offers the hope of improved basic understanding of the disease process as well as the possibility of novel, and perhaps more convenient, approaches to diagnosis.

Acknowledgements

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