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Widespread Histologic Distribution of the $\alpha_2\beta_1$ Integrin Cell-surface Collagen Receptor

Mary M. Zutter and Samuel A. Santoro
From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri

The $\alpha_2\beta_1$ integrin (platelet membrane glycoprotein Ia-IIa, VLA-2, ECMR-II) functions as a cell surface receptor for collagen. The authors have determined the histologic distribution of the $\alpha_2\beta_1$ receptor in normal tissues by immunohistochemical technique. The studies revealed that the $\alpha_2\beta_1$ receptor was expressed on fibroblasts, endothelial cells, and epithelial cells from multiple sites including skin, tonsil, breast, sweat gland, gastrointestinal tract, lung, bladder, cervix, and prostate. Follicular dendritic cells of the lymph node, tonsil, and spleen and dendritic cells of the thymus also expressed the $\alpha_2\beta_1$ receptor. The receptor also was present on Schwann cells of ganglia and on neuroglia. Greatly enhanced expression of the receptor in regions of proliferating epithelium suggests that enhanced expression of $\alpha_2\beta_1$ is associated with orderly, regulated cell proliferation. The circumferential staining pattern of the $\alpha_2\beta_1$ integrin within many epithelia is virtually identical to that observed for other adhesive receptors, such as the cadherins, which have been implicated in cell–cell adhesion. (Am J Pathol 137:113–120)

Recent studies in our laboratory, as well as in other laboratories, have established that the platelet membrane glycoprotein Ia-IIa complex serves as a cell surface receptor for collagen and mediates the Mg^{++}-dependent adhesion of platelets to types I, II, III, and IV collagen.[1–5] Liposomes containing the purified Ia-IIa complex have been shown to bind in a specific, Mg^{++}-dependent manner to substrates composed of collagen types I, II, III, and IV, but not to substrates of other adhesive proteins such as fibronectin, vitronectin, laminin, thrombospondin, von Willebrand factor, or fibrinogen.[3] Monoclonal antibodies directed against the Ia-IIa complex have been shown to inhibit the adhesion of platelets to collagen.[3–5]

The platelet membrane glycoprotein Ia-IIa complex is a member of the integrin superfamily of adhesive protein receptors, which have been shown to mediate the adhesive properties of many cell types and which may be involved in embryogenesis, differentiation, motility, and transformation.[6–8] Studies in our laboratory and in other laboratories have established the identity of the platelet membrane Ia-IIa complex with the VLA-2 (very late activation antigen-2) complex on T cells and with the ECMR-II (extracellular matrix receptor-II) on fibroblasts.[2–4,9,10] Current nomenclature assigns the designation $\alpha_2\beta_1$ to this receptor complex.[6]

The presence of the receptor on both activated T cells and on fibroblasts suggests that the adhesive mechanism mediated by the receptor is not unique to platelets but is shared by other cell types. Indeed, Wayner and Carter[11] have demonstrated that the same monoclonal antibody directed against the $\alpha_2\beta_1$ receptor that inhibits platelet adhesion to collagen also inhibits the adhesion of fibroblasts to collagen. We have undertaken the investigation described in this report to establish the tissue distribution of the $\alpha_2\beta_1$ cell surface collagen receptor in the human.

Materials and Methods

Antibodies

Monoclonal antibody 12F1, which is directed against the α_2 subunit of the $\alpha_2\beta_1$ integrin, was provided by Virgil Woods, University of California, San Diego. The characterization and specificity of this antibody have been previously described.[9,12] The Leu-4 (CD3) monoclonal antibody was obtained from Becton-Dickinson, Mountain View, California.

Immunohistochemistry

Tissue was obtained from material submitted to the surgical pathology service or the autopsy service of the Depart-

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Address reprint requests to Dr. Samuel A. Santoro, Department of Pathology, Box 8118, Washington University School of Medicine, St. Louis, MO 63110.

ment of Pathology, Washington University School of Medicine. The tissue was embedded in OCT compound (Miles Laboratories, Elkhart, IN), snap-frozen in liquid nitrogen-cooled isopentane, and then stored at −70°C. Frozen sections (6 μ) were cut, fixed briefly in acetone, and held at −20°C before staining by the immunoperoxidase technique using either the 12F1 or Leu-4 (CD3) reagents as the primary antibody, at a concentration of 5 μg/ml. The latter antibody served as a negative control for most cases of 12F1 positivity. Detection was achieved with biotinylated anti-mouse gamma G immunoglobulin (IgG) and avidin-biotin-peroxidase complex (Vector, Burlingame, CA) as previously described.[13]

Results

Sections of skin, including keratinizing squamous epithelium, dermis with adnexa, and subcutaneous fibroadipose tissue, were examined by the immunoperoxidase technique for expression of the $\alpha_2\beta_1$ integrin. Fibroblasts, as expected, and endothelial cells in the dermis expressed the receptor, although staining was of rather low intensity. Endothelial cell reactivity was manifest as a fine, linear pattern of staining that completely circled the vessels (Figure 1A). Endothelial expression of $\alpha_2\beta_1$ was confirmed by immunofluorescence analysis of cultured human endothelial cells.[14] Fibroblast immunoreactivity was distributed along the cytoplasmic processes of the cells (Figure 1B).

The basal cell layers of the epidermis were strongly positive for $\alpha_2\beta_1$ expression (Figure 2). The most intense staining was restricted to the deepest cell layer, the stratum germinativum. The second cell layer, the stratum spinosum, stained much less intensely. The more superficial layers, including the stratum granulosa and the keratinizing epithelium, did not stain. Intensity of staining of the basal cell layer was approximately four times that of endothelial cells and fibroblasts (Table 1).

Sweat glands within the deep dermis (Figures 2A, 3) also expressed the $\alpha_2\beta_1$ receptor. The intensity of staining of the basal layer (4+) of the bilayered cuboidal epithelium was greater than that of the superficial layer (2+). Staining was localized not only to the site of attachment to the basement membrane, but completely encircled the cell at all sites of cell–cell contact.

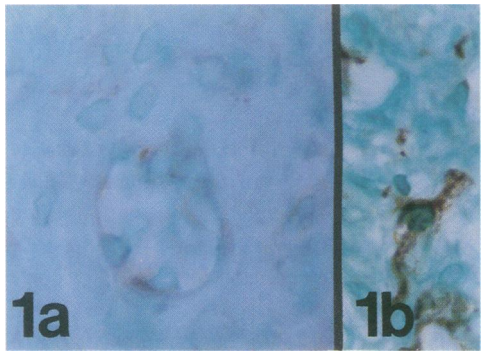
Evaluation of the gastrointestinal tract included examination of the stomach, ileum, colon, and liver. The gastrointestinal epithelium was positive for $\alpha_2\beta_1$ expression at all sites examined. The colonic epithelium revealed the most distinctive reaction pattern. Cells at the base of the crypts of Lieberkühn were strongly positive, with a gradation in staining intensity that diminished as the cells approached the colonic surface (Figure 4A). Individual cells showed a pattern of reactivity similar to that observed for other epithelial cells, ie, there was strong reactivity at the site of contact between cell and basement membrane. There also appeared to be an additional accentuation of staining at the apical aspect of the lateral cell surface at sites of cell–cell adhesion corresponding to the location of tight junctions (Figure 4B).

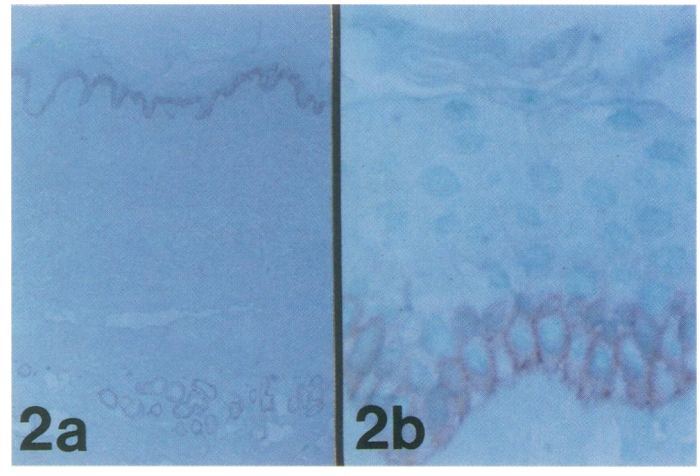
The epithelium of the small bowel also expressed $\alpha_2\beta_1$ in a distribution similar to that of the colon, but staining was less intense. Cells lining the glands were more strongly positive than were those covering the villi. The weaker staining of cells of the villi was most prominent at the basilar portions of the cells. The stomach differed markedly from other sites studied within the gastrointestinal tract. Only scattered epithelial cells (<2%) were faintly positive (trace to 1+) relative to the control antibody, as shown in Figure 5. This result was obtained in four independent experiments. Hepatocytes within normal liver failed to express the $\alpha_2\beta_1$ integrin to a level detectable by our technique.

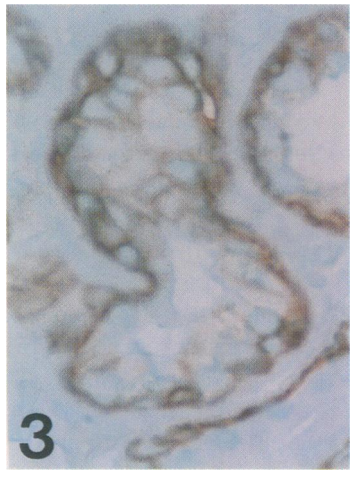
The large ducts, small ductules, and terminal duct units of the normal, nonlactating breast expressed the receptor (Figure 6). The staining was intermediate to intense (3 to 4+) in comparison with either the basal cell layer of the epidermis (4+) or the endothelial cells and fibroblasts (1+). Both the deep cuboidal epithelial cells and the superficial cuboidal or columnar epithelium were positive (Figure 6B). There was intense staining at the region of contact with the basement membrane and at sites of cell–cell contact, and weak, diffuse cytoplasmic staining. The distribution of the reactivity was similar to that observed in the sweat glands.

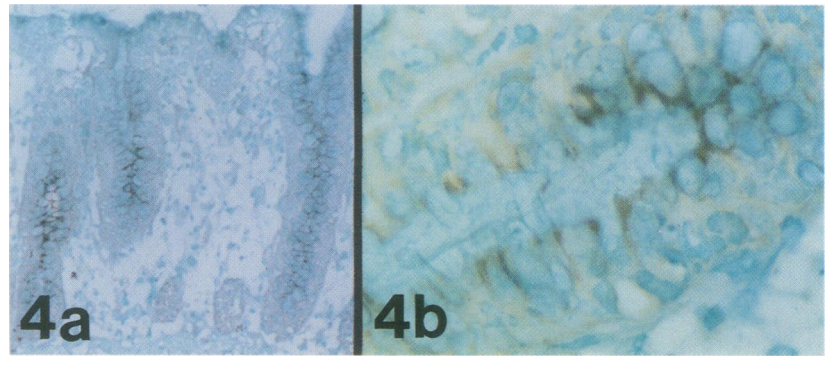
Multiple sites in the genitourinary tract expressed the receptor. The $\alpha_2\beta_1$ integrin was localized primarily to the renal tubules in the kidney (Figure 7). The intensity was 1 to 2+ when compared with the negative control. The endothelial cells of the glomeruli stained with low intensity, similar to that seen in endothelial cells throughout the body. The transitional epithelium of the bladder showed 2 to 3+ reactivity (Figure 8A). The staining intensity was greater in the basal cell layer. Brunn's nests, clusters of immature basal transitional epithelium believed to represent proliferative foci, were strongly positive (Figure 8B). The entire cell surface appeared as a distinct rim of positive staining.

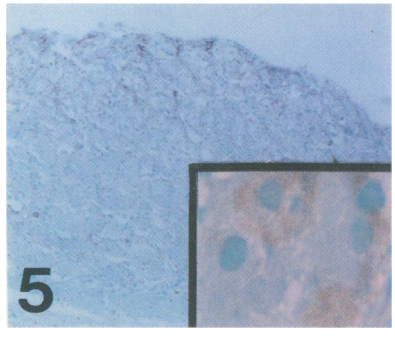
The prostatic glands showed intermediate (1 to 2+) reactivity. As shown in Figure 9, staining was most intense at the area of basement membrane contact. Intracellular reactivity, weaker than that observed in the breast, sweat gland, or bladder, also was seen. The endocervical glands were only weakly positive, with staining limited to the base of the glands at the site of attachment to the basal lamina (Figure 10). Other than the endothelial cells and fibroblasts, the endocervical stroma and bundles of

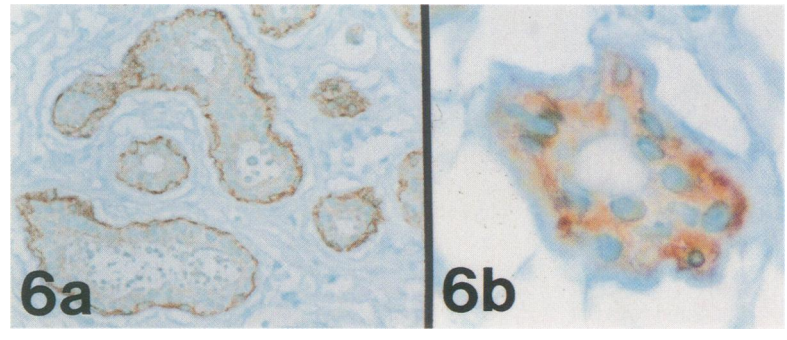


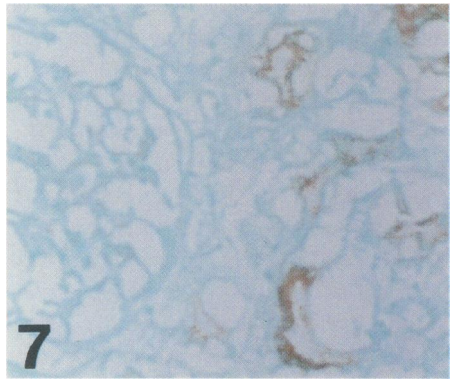












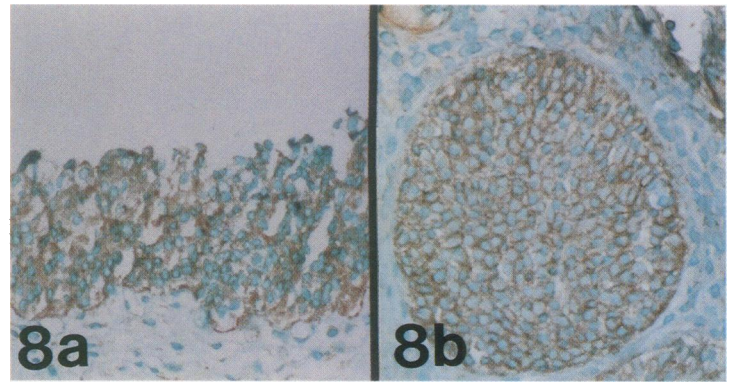


Figure 1. **A:** *Endothelial cells: Small vessels are lined by a weakly staining (1+) endothelium (×1000).* **B:** *Fibroblasts: Fibroblasts within the stroma of all tissues were weakly positive (×1000).* **Figure 2.** **A:** *Skin and subcutaneous tissues: the basal cell layer of the epidermis and the sweat gland epithelium strongly express the $\alpha_2\beta_1$ receptor (×100).* **B:** *Epidermis: the basal cell layer is strongly positive; the stratum spinosum stains weakly; the superficial keratinizing epithelium is negative (×1000).* **Figure 3.** *Sweat gland: both layers of the cuboidal epithelium express the receptor, however, expression is more intense in the basal layer (×1000).* **Figure 4.** **A:** *Colon: the mucosa reveals a distinct staining pattern with cells at the crypts of Lieberkuhn, being strongly positive; mucosal staining diminishes in the more superficial aspects of the glands (×100).* **B:** *Colonic gland: staining is accentuated at the apical aspect of the lateral cell surface (×1000).* **Figure 5.** *Stomach: The gastric mucosa contains only occasional faintly staining (trace − 1+) cells (×100) (inset, ×1000).* **Figure 6.** **A:** *Breast: Breast ducts and small ductules are strongly positive (×100).* **B:** *Breast duct: the basal layer stains most intensely; the superficial layer is positive but less intense (×1000).* **Figure 7.** *Kidney: the renal tubules show faint reactivity (×100).* **Figure 8.** **A:** *Bladder: all layers of transitional epithelium of the bladder express the receptor (×1000).* **B:** *Brunn's nest: The epithelial cells in Brunn's nests are strongly positive (×400).*

Table 1. *Histologic Distribution of the $\alpha_2\beta_1$ Integrin*

Cell type	Intensity	Location
Fibroblasts	1+	Processes
Endothelial cells	1+	Diffuse
Smooth muscle	—	
Skeletal muscle	—	
Peripheral nerve	—	
Neuron (cerebral cortex)	—	
Astrocytes	2+	Diffuse
Ganglion cells	—	
Schwann cells	4+	Diffuse
Lymphocytes		
Lymph node	—	
Tonsil	—	
Spleen	—	
Thymus	—	
Dendritic cell		
Follicular (lymph node, spleen, tonsil)	3+	Processes
Thymic	3+	Processes
Epithelium		
Keratinizing (skin)	4+	Basal cell layer
Nonkeratinizing	3-4+	All layers
Breast	4+	Intercellular junctions and basal
Sweat gland	4+	Intercellular junctions and basal
Stomach	trace—1+	Rare cells
Ileum	2-3+	Basolateral
Colon	3+	Basolateral and intercellular junctions
Cervix	1+	Basement membrane
Prostate	2+	Basement membrane, intercellular junctions
Bladder	2-3+	Intercellular junctions. Brunn's nests
Lung		
Ciliated columnar	3+	Intercellular border
Type II pneumocyte	3-4+	Diffuse

smooth muscle were negative. The myometrium was entirely negative for $\alpha_2\beta_1$ expression.

Hematopoietic tissues including tonsil, lymph node, thymus, and spleen revealed complex and intricate staining patterns. The nonkeratinizing squamous epithelium of the tonsil, in contrast to the keratinizing epidermis, expressed the receptor throughout the entire thickness of the epithelium, although a gradient of decreasing expression from the basal layer to the surface was apparent (Figure 11A). The epithelium lining the tonsilar crypts also expressed the $\alpha_2\beta_1$ integrin. Expression was largely restricted to the cell surface, with the cell borders sharply demarcated by an intense staining (Figure 11B).

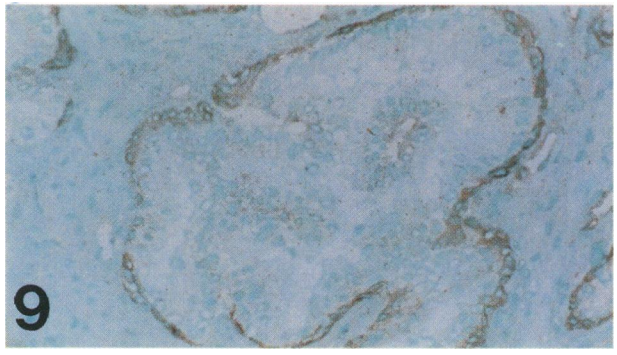
Lymphoid follicles in the tonsil and lymph nodes revealed similar patterns of staining (Figure 12A). Lymphocytes within the follicles were negative at the sensitivity of the technique employed. However, the follicles were distinctly outlined by a multilayered network of positively staining cells with fine delicate processes. Scattered cells with dendritic processes interlaced among lymphocytes within the follicles stained positively for $\alpha_2\beta_1$ (Figure 12B). Based on morphology, distribution, and the previously documented expression of other members of the β_1 family of integrins,[15] it is likely that these cells represent follicular dendritic cells. The lymphoid cells of the mantle zone and the interfollicular lymphoid cells were negative for $\alpha_2\beta_1$. In the interfollicular region, only the endothelial cells were weakly positive.

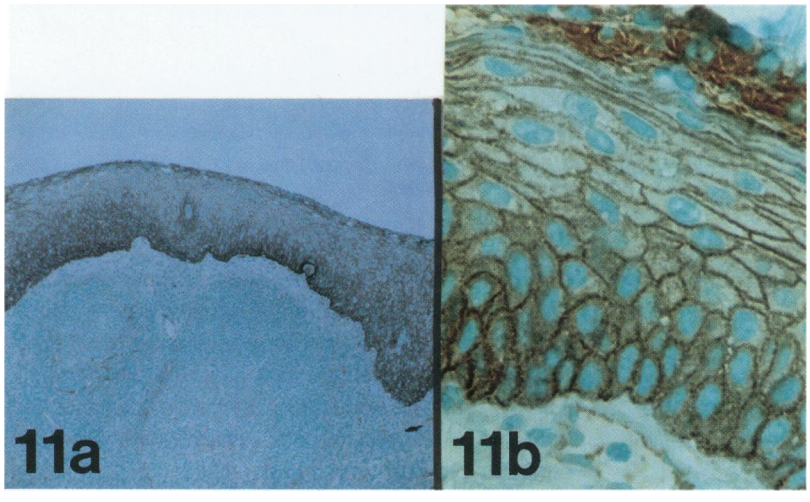
The thymic lobules were clearly demarcated by positively staining cells within the distribution of the connective tissue septa. The thymocytes were generally negative at the sensitivity of the technique, although occasional weak positivity (<1+) was observed. Throughout the lobules, positively staining cells with distinct interweaving processes were identified (Figure 13). Based on morphology and distribution it seemed likely that these cells corresponded to the follicular dendritic cells of the thymus.

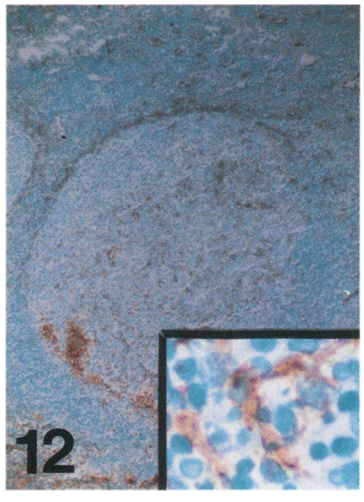
Sections of the spleen showed prominent staining of trabecula and the fibrous sheaths surrounding the trabecular and penicilliar arteries (Figure 14). This staining, although attributed to fibroblasts, was of greater intensity than observed in fibroblasts elsewhere. Lymphocytes within the follicles were negative, but the follicles were circumscribed by a network of strongly positive cells, as observed in lymphoid follicles elsewhere (Figure 12). The white pulp was weakly positive throughout the sinusoids. This reactivity was attributed to endothelial cells and fibroblasts, which form the backbone of the sinusoidal spaces. A rare lymphoid cell within the spleen stained positively for $\alpha_2\beta_1$. The identity of these cells could not be definitively established, however.

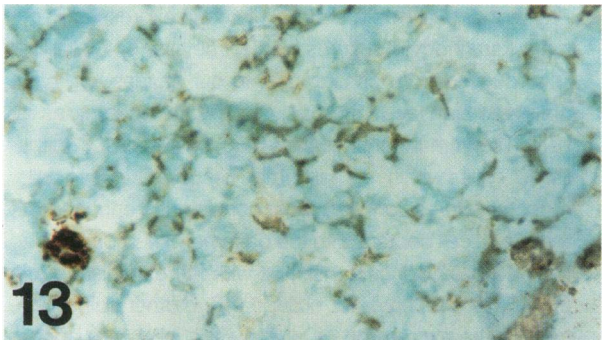
Study of the lung revealed a very complex pattern of staining (Figure 15). The ciliated columnar epithelium of bronchi and bronchioles stained positively (3 to 4+). Staining was most intense along, but not confined to, the basal and lateral aspects of the cells (Figure 15B). The alveolar septa revealed a fine 2+ pattern of linear staining that outlined the septal walls in a distribution corresponding to the capillary framework of the alveoli. A few larger cells lining the alveolar walls, with the appearance of type II pneumocytes, were also positive for $\alpha_2\beta_1$ expression. Not all type II pneumocytes, however, were positive. Those cells located at branch points of alveoli, which morphologically were more plump and epithelioid as opposed to flattened, appeared to be positive. It has previously been suggested that the proliferative activity of type II pneumocytes is confined to cells with a similar appearance.[16] The mesothelial lining of the pleura stained with 3+ positivity (Figure 15C).

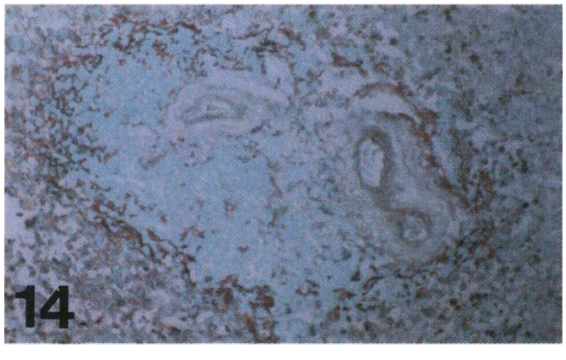
Study of ganglia of the myenteric plexus of the gastrointestinal tract revealed that the ganglion cell bodies did











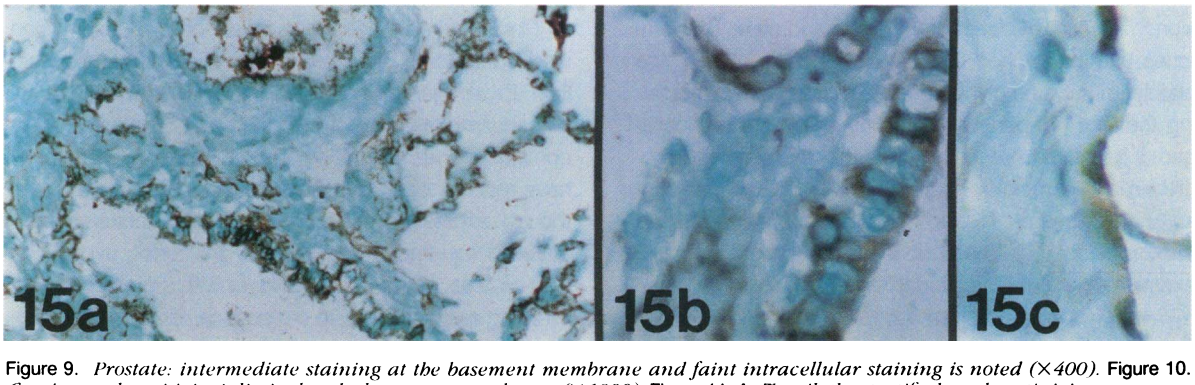
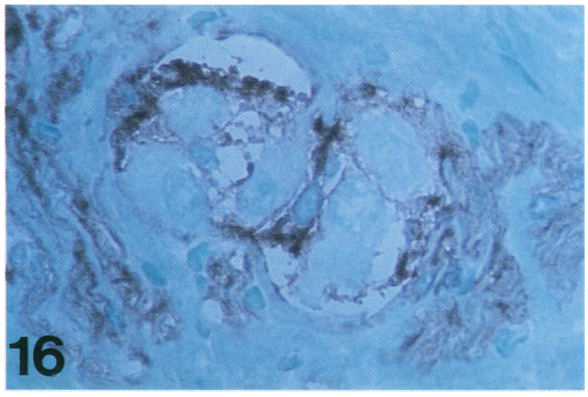


Figure 9. *Prostate: intermediate staining at the basement membrane and faint intracellular staining is noted (×400).* Figure 10. *Cervix: weak positivity is limited to the basement membrane (×1000).* Figure 11. A: *Tonsil: the stratified nonkeratinizing squamous epithelium is strongly positive throughout the entire thickness (×100).* B: *Tonsillar epithelium: Expression is most intense at the cell surface (×1000).* Figure 12. A: *Lymphoid follicle: lymphocytes are negative; a fine network of positivity outlines the follicles (×100).* B: *Germinal center: positively staining follicular dendritic cells interlace between nonreactive lymphoid cells (×1000).* Figure 13. *Thymus: dendritic cells are positive; thymocytes are negative (×1000).* Figure 14. *Spleen: the trabecula and fibrous sheaths are strongly positive (×100).* Figure 15. A: *Lung: ciliated columnar epithelium, alveolar septa, and scattered type II pneumocytes are positive (×100).* B: *Bronchi: the ciliated columnar epithelium is positive for $\alpha_2\beta_1$ expression (×1000).* C: *Pleura: the pleural mesothelium is positive (×1000).*



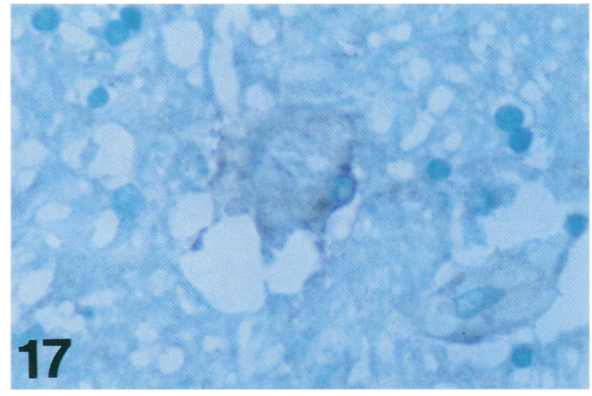


Figure 16. *Ganglia: Schwann cells show strong reactivity; ganglia cell bodies are negative (×1000).* **Figure 17.** *Cerebral cortex: fine, granular positivity is noted throughout; large astrocytes show increased staining (×1000).*

not express $\alpha_2\beta_1$, but that Schwann cells and Schwannian processes strongly expressed (4+) the receptor (Figure 16). Examination of peripheral nerve revealed no expression by the nerve itself. Studies of the cerebral cortex revealed a fine, granular 1 to 2+ positivity throughout (Figure 17). Large astrocytes were more strongly positive (2 to 3+).

Skeletal muscle and smooth muscle within the gastrointestinal and genitourinary tracts were entirely negative. Smooth muscle bundles within the walls of large vessels also were negative. In these studies, the presence of fibroblasts and endothelial cells served as useful internal positive controls.

Discussion

The integrin superfamily of adhesive protein receptors encompasses a growing number of heterodimeric receptors that mediate both cell–substrate and cell–cell adhesion.[6–8] Recent evidence from this[1–3] and other[4,5] laboratories has established the role of the $\alpha_2\beta_1$ integrin, the platelet membrane glycoprotein Ia-IIa complex, in mediating the Mg^{++}-dependent adhesion of platelets to collagen. It has also been established that the platelet glycoprotein Ia-IIa complex is identical to the VLA-2 antigen originally described on T cells[2,9] and to the ECMR-II defined on fibroblasts.[3,4,10] These observations prompted us to examine other cell types and tissues for expression of the $\alpha_2\beta_1$ cell surface collagen receptor. Expression of the $\alpha_2\beta_1$ integrin was studied with the 12F1 monoclonal antibody using immunohistochemical techniques. We have previously established that this antibody reacts with but does not inhibit the collagen-binding activity of the $\alpha_2\beta_1$ integrin.[2] Hence, occupancy of the receptor with ligand is unlikely to impair staining.

The receptor has a widespread histologic distribution, suggesting an important role for the $\alpha_2\beta_1$ integrin in mediating the adhesive properties of diverse cell types. The cell and tissue distribution of the receptor is summarized in Table 1. The $\alpha_2\beta_1$ receptor was expressed on fibroblasts and endothelial cells throughout the body. Expression by fibroblasts was anticipated from studies reported previously.[11,12] During the course of this investigation, two other groups reported expression of the $\alpha_2\beta_1$ integrin by endothelial cells.[17,18]

Wayner et al[19] detected $\alpha_2\beta_1$ integrin expression in primary keratinocyte cultures and on several epithelial cell lines derived from carcinomas. In our studies, we have observed high levels of expression of the receptor, as revealed by immunochemical staining, on numerous epithelial cell types within normal human tissues. On these cells, receptor expression was most consistently enhanced at the site of cell contact with the basement membrane. The basilar localization is consistent with the established role of $\alpha_2\beta_1$ as a receptor for collagen. Two other points concerning the expression of $\alpha_2\beta_1$ by epithelial cells appear to be especially cogent.

First, markedly enhanced levels of $\alpha_2\beta_1$ expression were consistently observed at sites of proliferating epithelium. Expression of the receptor in the keratinizing stratified squamous epithelium of skin was almost exclusively confined to the proliferating basal cell layer. Similar results have recently been described for human fetal skin.[19] Although expressed by all layers of the nonkeratinizing stratified squamous epithelium of the tonsil, expression was greatest in the basal cell layer with a gradient of decreasing expression up to the surface. Similarly in the colon, a gradient of decreasing expression of $\alpha_2\beta_1$ was observed from the proliferating cells located at the base of the crypts of Lieberkühn to the colonic surface. The expression of $\alpha_2\beta_1$ also was greatly enhanced within Brunn's nests, foci of proliferating epithelial cells within the bladder wall. Expression of the receptor by alveolar type II pneumocytes was increased in cells thought to represent proliferating type II pneumocytes.[16] Hemler and

colleagues[20,21] have previously described the increased expression of $\alpha_2\beta_1$ (VLA-2) on cultures of T cells stimulated with mitogen, antigen, or antibody against T3. These observations suggest that the $\alpha_2\beta_1$ receptor may play a role in orderly, regulated cellular proliferation. Additional studies are obviously required to establish this point.

Second, in many tissues, $\alpha_2\beta_1$ expression was enhanced at sites, which suggest that it may function as a cell–cell adhesion molecule, in addition to its established role in cell–substrate adhesion. For example, in stratified squamous epithelia, staining revealed the receptor to be circumferentially distributed about the cell at all points of cell–cell contact. Within the colon, enhanced expression (and, in fact, the site of most intense staining) was localized to the apicolateral aspect of the cells at sites corresponding to the location of tight junctions. Similarly, within the Brunn's nests of the bladder, intense circumferential staining completely outlined the cell surface. We cannot exclude the possibility that the $\alpha_2\beta_1$ in these locations is simply vestigial receptor remaining from a time when these cells were in contact with a basement membrane. Receptor unoccupied with ligand may be free to diffuse about the cell surface. However, the highly localized distribution of the receptor to discrete regions of the cell surface, as in the colon, makes this possibility less likely. We likewise cannot exclude the possibility that the receptor is bound to small amounts of a collagen, especially one of the many types recently defined whose function remains to be established. With regard to the possible role of $\alpha_2\beta_1$ in cell–cell adhesion, it is noteworthy that recent determination of a cDNA sequence for the α_2 subunit revealed the presence of an I domain.[22] Within the superfamily of integrins, this domain was previously thought to be confined to the α subunits of members of the β_2 family, all of which function as leukocyte cell–cell adhesion molecules.[6,23]

A comparison of the distribution of the $\alpha_2\beta_1$ integrin with the distribution of other adhesion receptor proteins for which extensive information exists reveals several similarities, as well as differences. The distribution of $\alpha_2\beta_1$ is in many respects similar to that reported for E-cadherin (uvomorulin, L-CAM, Arc-1, cell CAM 120/80), a non-integrin Ca^{++}-dependent cell adhesive molecule that mediates cell–cell interactions.[24] Although the distribution of $\alpha_2\beta_1$ and E-cadherin within most epithelia appear to be similar, E-cadherin, unlike $\alpha_2\beta_1$, is expressed only by epithelial cells. Hepatocytes strongly express E-cadherin, whereas our results suggest that normal hepatocytes express little, if any, $\alpha_2\beta_1$. The distribution of $\alpha_2\beta_1$ within the stratified squamous epithelium of skin appears to be virtually identical to that recently reported for the $\alpha_E\beta_4$ integrin.[25] Like E-cadherin, the expression of $\alpha_E\beta_4$ appears to be confined to epithelial cells. The concordance between the tissue distribution of the $\alpha_2\beta_1$ integrin and that of the adhesive cell surface proteoglycan described by Bernfield and colleagues[26] is striking. The functional significance of this concordance is unknown.

In summary, the $\alpha_2\beta_1$ integrin is expressed on a diverse array of cell types in numerous tissues. The subcellular sites of expression suggest that the molecule may function not only in cell–substrate adhesion, where it may play a role in orderly, regulated cell proliferation, but that the receptor may also play a significant role in cell–cell adhesion.

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