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line HL-60 were also stained by these antibodies. Immunohistochemistry on colon sections using the antibodies in this Workshop were positive on iIEL [Rizzo *et al.*, unpublished Workshop report].

Function

The $\alpha^E\beta_7$ integrin mediates adhesion between iIEL T-cell lines and epithelial cells *in vitro* [7–9]. *In vivo*, iIEL are localized to the basolateral surface of epithelial cells and *in vitro* co-culture of iIEL and epithelial cells can reconstitute this localization [7]. Thus, iIEL may utilize $\alpha^E\beta_7$ to recognize a cellular ligand specifically expressed on the basolateral surface of epithelial cells. E-cadherin is one such molecule and as anti-E-cadherin mAb inhibit IEL-to-epithelial cell adhesion *in vitro*, E-cadherin is a candidate counter receptor for $\alpha^E\beta_7$ [10]. With the cloning of CD103 and the search for the ligand underway, more information will soon become available about this unique site-specific integrin α chain.

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AS7/8.5 CD104 (β_4) cluster report

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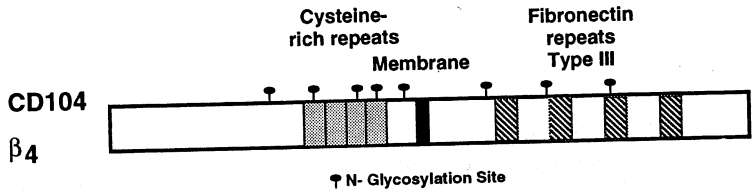

The CD104 cluster designation was assigned in this Workshop to the integrin β_4 subunit. β_4 associates with the α^6 chain, which also associates with the β_1 integrin chain [1]. Both $\alpha^6\beta_1$ and $\alpha^6\beta_4$ appear to bind laminin, although there may be other ligands [2]. Three monoclonal antibodies (mAb), S235 (UM-A9), S247 (439-9B), and S248 (450-11A1), were used in blinded studies to cluster this antigen. S248 (450-11A1), which has been reported to be against a cytoplasmic epitope, did not cluster well in flow cytometry studies but it does immunoprecipate β_4. S235 (UM-A9), S247 (439-9B), and S205 (AA3) in Subpanel 6 were confirmed to be specific for β_4 in studies on transfected cells [Hemler *et al.*, AS6.7]. S248 (450-11A1) was not reactive with β_4 transfectants by flow cytometry, consistent with a cytoplasmic location of its epitope.

Molecular cloning

The β_4 cDNA [3,4] is unique among integrin β chains because it has a long cytoplasmic tail of 1000 residues (introductory diagram). The extracytoplasmic region shows homology to the other β integrins, but there is poor homology in the transmembrane region and no integrin counterpart for the cytoplasmic tail. The long cytoplasmic tail includes four repeats that are homologous to the type III repeats in fibronectin. The gene is located on chromosome 17q11–qter [6].

Immunochemistry

In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) the β_4 chain migrates as a 220-kDa band under non-reducing and as a 205-kDa band under reducing conditions [7,8]. Two fragments of 180 and 150 kDa that are thought to be proteolytic cleavage products can independently associate with α^6 [7]. The associated α^6 chain runs as a 125/25-kDa cleaved product. All three mAb clustered to CD104 immunoprecipated a 205-kDa band from freshly isolated keratinocytes [Staquet, M. and Schmitt, D, unpublished Workshop report] and a 220-kDa non-reduced band from A439 cells (adenocarcinoma) [Bodorova and Hemler, unpublished Workshop report].

Cellular expression

β_4 expression has been reported on epithelia, Schwann cells, some tumour cells [9], some endothelia, neuronal cells, and the trophoblast [10]. Typically, $\alpha^6\beta_4$ is found on the basal surface of cells in contact with basement membranes [8,11] or at points of cell–cell contact, including the hemidesmosome. In flow cytometry and immunohistochemistry studies, CD104 was found to be expressed in epithelial cells of breast, colon, lung, thymus, tonsil, and skin, especially along the basement membrane. Human umbilical vein endothelial cells and endothelial cells in the tissue sections expressed CD104. The B-cell lines JY and NAD-20, monocytes, the myelocytic cell line HL-60, and colon carcinoma cells also expressed β_4. On HL-60 and monocytes, S248 (450-11A1) was reactive by flow cytometry.

Function

In association with α^6, CD104 has been shown to bind laminin [2], and epiligrin [12]. $\alpha^6\beta_4$ probably has a special role in cell attachment to the basement membrane and the hemidesmosome [13]. It has also been implicated in wound healing [14] and in T-cell and NK-cell cytotoxity [15]. S235 (UM-A9) has been reported previously to partially block laminin binding.

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