AS7/8.4 CD103 (α^E) cluster report

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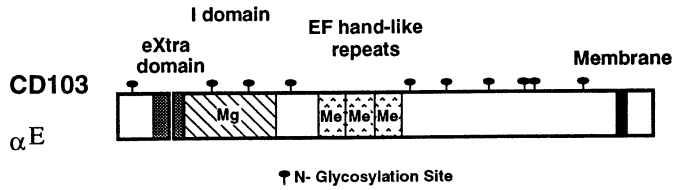

The CD103 antigen was first characterized by a monoclonal antibody (mAb) to human mucosal lymphocyte antigen 1 (HML-1) on intestinal intraepithelial lymphocytes (iIEL) [1]. CD103, the integrin α^E subunit, is part of a heterodimer containing the integrin β_7 subunit [2]. The β_7 integrin subfamily has two members, $\alpha^4\beta_7$ and $\alpha^E\beta_7$. Seven mAb clustered to CD103 in this Workshop: five antibodies from the Adhesion Panel, S237/S206 (F3F7), S238/S170 (F4F1), S242/A032 (LF61), S256 (Ber-ACT 8), and S257 (HML-1), and two mAb from the Activation Panel A005 (2G5.1) and A129 (B-LY7).

Molecular cloning

The gene encoding CD103, α^E, was isolated from a cDNA library synthesized from iIEL treated with transforming growth factor-β1 (TGF-β1) [3]. The predicted amino acid sequence revealed a type I transmembrane protein 1160 residues long (introductory diagram). Like other integrin α subunits, α^E contained seven repeated domains, an I domain, three potential divalent-cation-binding sites, and a GFFKR amino acid sequence in the cytoplasmic tail. Unlike other integrin α chains, α^E contained a region of 55 amino acids N-terminal to the I domain that had no homology to other integrin α chains. This unique eXtra (X) domain contained an unusual highly charged region and a dibasic proteolytic cleavage site. The location of the cleavage site near the N-terminus of the molecule was unique among integrin α subunits. The α^E amino acid sequence was most closely related in overall sequence to other I domain-containing integrins. However, α^E is the only integrin α chain that contains an I domain and undergoes post-translational proteolytic cleavage.

Immunochemistry

CD103 mAb immunoprecipitate a protein complex composed of two predominant chains of 105 kDa (β_7) and 175 kDa (α^E) under non-reducing conditions. Upon reduction, the 175-kDa chain dissociates to reveal two fragments of 150 and 25 kDa. The shift of CD103 upon reduction into two subunits suggests that the posttranslationally cleaved components remain associated by disulfide linkage. Pulse chase analysis reveals that the 150- and 25-kDa fragments of the α^E chain result from the cleavage of a precursor polypeptide during biosynthesis [4]. The five Workshop mAb in the Adhesion Panel were all able to immunoprecipitate $\alpha^E\beta_7$ from iIEL [Wong and Springer, AS7/8].

Cellular expression

The $\alpha^E\beta_7$ antigen is expressed on the surface of >95 per cent of iIEL and 40 per cent of lamina propria T lymphocytes in the intestine but <2 per cent of resting peripheral blood lymphocytes (PBL) [1]. $\alpha^E\beta_7$ is an activation antigen as *in vitro* stimulation of PBL with phytohaemagglutinin (PHA) induces expression [5]. $\alpha^E\beta_7$ expression is also regulated by TGF-β1. Incubation of iIEL *in vitro* with 2 ng/ml TGF-β1 causes a dramatic increase in both the percentage of iIEL expressing $\alpha^E\beta_7$ and in the level of expression [4]. $\alpha^E\beta_7$ can also be expressed on T-cell lymphomas [6] and B cells under special circumstances such as on hairy cell leukaemic splenocytes [4]. Flow cytometry during this Workshop showed that all five mAb in the Adhesion Panel stain iIEL cultured in TGFβ-1 in the biphasic pattern expected. Activated lymphocytes, some monocytes, and the myelocytic cell

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.

References

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AS7/8.5 CD104 (β_4) cluster report

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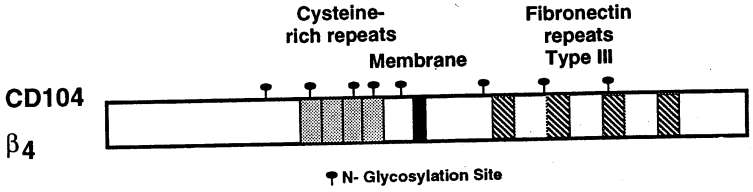

The CD104 cluster designation was assigned in this Workshop to the integrin β_4 subunit. β_4 associates with the α^6 chain, which also associates with the β_1 integrin chain [1]. Both $\alpha^6\beta_1$ and $\alpha^6\beta_4$ appear to bind laminin, although there may be other ligands [2]. Three monoclonal 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.