AS7/8.6 Integrin β_7 pre-CD report

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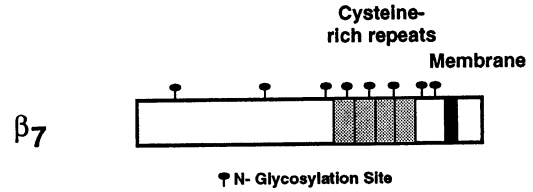

β_7 is an integrin β chain that associates with two α chains, α^E and α^4, to form a heterodimer involved in cell adhesion on the cell surface [1]. Two β_7 specific monoclonal antibodies (mAb) S253 (BP6) and S254 (ACT-1) were submitted to this Workshop. Studies during this Workshop confirmed that both mAb probably bind an epitope on the β_7 chain, but differences in the reactivities of the two mAb prevent the clustering of this antigen at this Workshop.

Molecular cloning

The cDNA clone of β_7 [2,3] shows that it is a type I transmembrane protein and has a structure typical of integrin β chains (introductory diagram). The sequence is most homologous to the β_2 integrin chain. The gene has been mapped to chromosome 12q13.13 [4].

Immunochemistry

The molecule runs as a 105-kDa band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions and as a 120-kDa band under reducing conditions [5]. Both antibodies submitted to the Workshop were found by Cepek and Brenner to immunoprecipitate $\alpha^E\beta_7$ from surface-iodinated intestinal intraepithelial lymphocytes (iIEL) [Wong and Springer, AS7/8, Fig. 2]. $\alpha^4\beta_7$ was also immunoprecipitated from peripheral blood mononuclear cells by mAb S254 (ACT-1) [Krissansen and Print, unpublished Workshop report].

Cellular expression

The expression of β_7 integrins, like β_2 integrins, appears to be limited to leucocytes [6]. The $\alpha^4\beta_7$ and $\alpha^E\beta_7$ integrins are expressed on distinct subsets of lymphocytes. The $\alpha^4\beta_7$ integrin has been shown to be involved in the homing of lymphocytes to Peyer's patch high endothelial venules in the gut [7]. Mucosal iIEL lack expression of $\alpha^4\beta_7$ and express $\alpha^E\beta_7$ [8]. Flow cytometry during this Workshop showed that mAb S253 (BP6) and S254 (ACT-1) have distinct staining patterns. S254 (ACT-1) stained T-cells, B-cells, monocytes, and NK cells, consistent with known $\alpha^4\beta_7$ expression. It did not stain iIEL cells. S253 (BP6) stained some Sézary cell leukaemias, like CD103 mAb, but in contrast, did not stain iIEL in flow cytometry. However, in immunohistochemistry, both S253 (BP6) and S254 (ACT-1) stained intraepithelial lymphocytes. The expression data along with the immunoprecipitation results suggest that S254 (ACT-1) is specific for the $\alpha^4\beta_7$ complex as previously reported [9] on intact cells, but can recognize $\alpha^E\beta_7$ after solubilization. S253 is an antibody to β_7 or the $\alpha^E\beta_7$ complex, but is directed towards an epitope that is exposed on the β_7 chain only in certain malignant cells or after cell permeabilization or solubilization.

Function

MadCAM-1 has been shown to be a ligand for $\alpha^4\beta_7$ and the interaction between MadCAM and $\alpha^4\beta_7$ appears to be important for homing of lymphocytes to the intestinal tract [10]. The ligand for $\alpha^E\beta_7$ is not yet known but one counterreceptor appears to be localized to the basolateral surface of gut epithelial cells [11,12].

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AS7/8.7 A new mAb defines a protein complex associated with the $\alpha^E\beta_7$ integrin

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An integrin made of the β_7 chain and of the α^E (CD103) chain has recently been identified at the surface of human intestinal intraepithelial lymphocytes (iIEL) [1–3]. This integrin was initially identified by the monoclonal antibody (mAb) S257 (HML-1) [4]. Another antibody directed against the same protein, HML-4 [5], induced a strong homotypic aggregation of human iIELs as well as of the $\alpha^E\beta_7$+MOLT-16 cell line (a gift from Dr Minowada, Fujisaki Cell Centre). HML-4-induced homotypic aggregation was independent of any increase in the density of membrane-associated $\alpha^E\beta_7$ integrin and had all the characteristics of an active adhesion event mediated via $\alpha^E\beta_7$. Indeed, it required an active metabolism and intact cytoskeleton; it was not inhibited by antibodies blocking identified lymphocyte adhesion pathways, CD11a, ICAM-1, -2, -3, CD2, CD58, CD29, CD29d, VCAM-1, or CD44. In contrast, it was blocked by three different antibodies directed against the $\alpha^E\beta_7$ integrin, mAb S257 (HML-1), S238 (F4F1 or HML-2), and S237 (F3F7 or HML-3) [1,2]. By analogy with comparable observations made with mAb directed against other integrins, these data suggested to us that HML-4 induced a conformational change of $\alpha^E\beta_7$ resulting in its higher binding avidity for a ligand coexpressed on the same cells. In order to identify the ligand of $\alpha^E\beta_7$ involved in homotypic aggregation or molecules able to interfere with the homotypic binding, new mAb were raised against the $\alpha^E\beta_7$+MOLT-16 cell line and selected for their ability to inhibit HML-4-induced homotypic aggregation.

One of the blocking mAb thus obtained, 6G4, immunoprecipitated from MOLT-16 cells and iIELs a protein complex made of four major polypeptides under reducing conditions (Fig. 1, lane 3). Two bands of 120 and 150 kDa were the β_7 and α^E chains of the integrin, respectively, as they could be exhausted from the lysates by prior immunoprecipitation with the antibody S257 (HML-1) (Fig. 1, lane 2). The two other bands correspond to two distinct proteins of 102 and 135 kDa under reducing conditions (Fig. 1, lane 4 and not shown). Only the 135-kDa band was directly recognized by mAb 6G4, as indicated by immunoprecipitation in the presence of sodium dodecyl sulfate (data not shown). Membrane immunofluorescence studies and immunohistochemical studies on tissue sections using mAb 6G4 showed a distribution of the 135-kDa protein strictly identical to that of the $\alpha^E\beta_7$ integrin. Altogether these results indicate that the new