

platelets, oestoclasts, mast cells, and tumour cells [10,11]. In association with β_5 , it is found on carcinoma cells, hepatoma cells, and fibroblasts [2,3]. Two cell lines, FG-2 and UCLA-P3, have been found to express $\alpha^V\beta_6$ [5]. During the Workshop, CD51 was found to be expressed in all stromal cells including fibroblastic, epithelial, hepatocytic, mesothelial, and endothelial cell lines. It was also present on JY, monocytic, and HMC-1 (mast cell) cells and weakly expressed on platelets. Immunohistochemistry showed staining in all organs, on epithelium, endothelium, fibroblasts, smooth muscle cells, and nerve tracts. While CD51 antibodies stain colon carcinoma and breast epithelial cells, CD51/CD61 complex antibodies did not stain these cells. These results suggest that α^V is associating with another β chain in those cells, probably β_5 .

Function

The $\alpha^V\beta_3$ integrin complex binds vitronectin at the RGD sequence and is also known to bind fibrinogen, von Willebrand factor (vWF), thrombospondin, fibronectin, osteopontin, and collagen [1]. While $\alpha^V\beta_5$ has also been shown to bind vitronectin at the RGD site, $\alpha^V\beta_1$ and $\alpha^V\beta_6$ have only been shown to bind fibronectin [2-5]. As cell surface molecules, the α^V molecules appear to have become the targets of pathogens. $\alpha^V\beta_3$ and $\alpha^V\beta_5$ have been shown to promote adenovirus internalization but not virus attachment [12]. $\alpha^V\beta_5$ has been shown to bind to the Tat protein of HIV [13]. *Mycobacterium avium-intracellulare* may use $\alpha^V\beta_3$ to invade monocytes [14]. During this Workshop, S236 (AMF7) was shown to block macrophage recognition of apoptotic cells. [Flora and Gregory, AS7.9]. S262 (LM142) and S263 (23C6) inhibited T-cell proliferation to tetanus

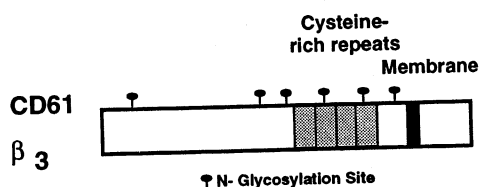
toxin peptide and allogeneic cells [Wyss-Coray and Pichler, unpublished Workshop report]. S236 (AMF7), S245 (13C2), S246 (23C6), and S263 (LM609) are reported by their donors to inhibit binding in adhesion assays but this was not tested during the Workshop.

References

1. Hynes, R. O. *Cell* **69**, 11 (1992).
2. Pytela, R., Pierschbacher, M. D., and Ruoslahti, E. *Proc. natl Acad. Sci., USA* **82**, 576 (1985).
3. Cheresch, D. A., Smith, J. W., Cooper, H. M., and Quaranta, V. *Cell* **57**, 59 (1989).
4. Vogel, B. E., Tarone, G., Giancotti, F. G., Gailit, J., and Ruoslahti, E. *J. biol. Chem.* **265**, 5934 (1990).
5. Busk, M., Pytela, R., and Sheppard, D. *J. biol. Chem.* **267**, 5790 (1992).
6. Moyle, M., Napier, M. A., and McLean, J. W. *J. biol. Chem.* **266**, 19650 (1991).
7. Suzuki, S., Argraves, W. S., Arai, H., Languino, L. R., Pierschbacher, M. D., and Ruoslahti, E. *J. biol. Chem.* **262**, 14080 (1987).
8. Sosnoski, D. M., Emanuel, B. S., Hawkins, A. L., van Tuinen, P., Ledbetter, D. H., Nussbaum, R. L., Kaos, F. T., Schwartz, E., Phillips, D., Bennett, J. S., et al. *J. clin. Invest.* **81**, 1993 (1988).
9. Fernandez-Ruiz, E., Pardo-Manuel de Villena, F., Rodriguez de Cordoba, S., and Sánchez-Madrid, F. *Cytogenet. Cell Genet.* **62**, 26 (1993).
10. Horton, M. *Int. J. exp. Pathol.* **71**, 741 (1990).
11. Guo, C. B., Kagey-Sobotka, A., Lichtenstein, L. M., and Bochner, B. S., *Blood* **79**, 708 (1992).
12. Wickham, T. J., Mathias, P., Cheresch, D. A., and Nemerow, G. R. *Cell* **73**, 309 (1993).
13. Vogel, B. E., Lee, S. J., Hildebrand, A., Craig, W., Pierschbacher, M. D., Wong-Staal, F., and Ruoslahti, E. *J. Cell Biol.* **121**, 461 (1993).
14. Rao, S. P., Ogata, K., and Catanzaro, A. *Infect. Immun.* **61**, 663 (1993).

AS7/8.3 CD61 (β_3) cluster report

DENNIS A. WONG and TIMOTHY A. SPRINGER



CD61 is the integrin β_3 chain that was initially discovered as part of the heterodimer gpIIb/IIIa (CD41/CD61), a major glycoprotein found on platelets [1]. With the cloning of CD41/CD61 and the finding that the vitronectin receptor is $\alpha^V\beta_3$ (CD51/CD61), it became clear that CD41/CD61 and

CD51/CD61 are members of the β_3 subfamily of the integrins [2]. CD61 was clustered at the Fourth Workshop with four monoclonal antibodies (mAb) Y2/51, CLB-thromb/1, VI-PL2, and BL-E6. Thirteen mAb have been clustered to CD61 in this Workshop: five antibodies from Adhesion Structure Subpanel 7, S234 (7G2), S239 (CLB-Thromb/1), S249 (AP5), S250 (AP6), and S258 (C5-1); one mAb S126 (7E3) from Adhesion Structure Subpanel 5, which was not included in many studies conducted on other mAb; six mAb from the Platelet Panel, P015 (A3.A10), P022 (Y-2/51), P051 (RUU-PL7F12), P088 (Sz.21), P093 (P3-13), and P096 (PM6/13); and one mAb T/124 (VI-PL2) from the T-cell Panel. Although the five mAb in Subpanel 7 bind to the β_3 chain, two mAb S239 (CLB-thromb/1) and S249 (AP5), appear to prefer CD41/CD61 over CD51/CD61. S250 (AP6) and S234 (7G2) bind to only a subset of CD61 molecules. S258 (C5-1) is a rat antibody that binds to both CD41/CD61 and CD51/CD61 by flow cytometry and immunoprecipitation and was not detected well with anti-mouse reagents.

Molecular cloning

CD61 is a type I transmembrane protein with a typical integrin β -chain structure [3] (introductory diagram). CD61 maps to chromosome 17q21-23 [3].

Immunochemistry

The protein runs at 90 kDa as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and at 105 kDa under non-reducing conditions [4]. CD41/CD61 was immunoprecipitated from surface-iodinated platelets by the five CD61 mAb in Subpanel 7, with S239 being weaker than the others [Wong and Springer, AS7/8]. Of these mAb, only S258 and S234 precipitated CD51/CD61 from JY cells. S239 and S249 were able to immunoprecipitate weak bands of CD51/CD61 from osteoclasts [Nesbitt and Horton, AS7/8.19].

Cellular expression

CD41/CD61 ($\alpha^{IIb}\beta_3$) is expressed almost exclusively on platelets and megakaryoblasts [1]. CD51/CD61 ($\alpha^V\beta_3$), the vitronectin receptor, is found on endothelial cells, some B cells, monocytes/macrophages, platelets, osteoclasts, mast cells, and tumour cells [5,6]. By flow cytometry, CD61 was found on platelets, monocytic cells, some B-cell lines, endothelium, and almost all stromal cell lines. S234 (7G2), S250 (AP6), and S258 (C5-1) stained platelets poorly compared to S239 (CLB-thromb/1) and S249 (AP5), with S250 (AP6) staining activated platelets well and reacting with some CD51/CD61 positive cells.

Function

In combination with both CD41 (α^{IIb}) and CD51 (α^V), CD61 appears to bind to a number of extracellular proteins through recognition of the RGD sequence [5]. (See CD41 [Honda *et al.*, P7.1 and CD51 [Wong and Springer, AS7/8.2] reports for more information on specific heterodimer function.) S250 (AP6) binds to an activation epitope on CD41/CD61 and binds CD51/CD61 as well, and may be useful in monitoring activation of both heterodimers.

References

1. Phillips, D. R., Charo, I. F., and Scarborough, R. M. *Cell* **65**, 359 (1991).
2. Hynes, R. O. *Cell* **69**, 11 (1992).
3. Lanza, F., Kieffer, N., Phillips, D. R., and Fitzgerald, L. A. *J. Biol. Chem.* **265**, 18098 (1990).
4. Hemler, M. E. *Ann. Rev. Immunol.* **8**, 365 (1990).
5. Horton, M. *Int. J. exp. Pathol.* **71**, (1990).
6. Guo, C. B., Kagey-Sobotka, A., Lichtenstein, L. M., and Bochner, B. S. *Blood* **79**, 708 (1992).
7. Smith, J. W., Ruggeri, Z.M., Kunicki, T. J., and Cheresch, D. A. *J. Biol. Chem.* **65**, 12267 (1990).
8. Steiner, B., Cousot, D., Trzeciak, A., Gillissen, D., and Hadvary, P. *J. Biol. Chem.* **264**, 13102 (1989).
9. Plow, E. F. and Ginsberg, M. H. *Prog. Hemostas. Thrombos.* **9**, 117 (1989).