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CLONING OF A NOVEL PROTEIN THAT INTERACTS WITH THE ALPHA 5 INTEGRIN SUBUNIT ((S. K. Alahari, J. W. Lee, and R. L. Juliano.)) Department of Pharmacology, University of North carolina, School of Medicine, Chapel Hill, NC 27599-7365

Recently, we observed that ectopic expression of $\alpha 5$ subunit in human carcinoma cells reduces their ability to form tumors in nude mice. Another piece of data from our laboratory indicated that the expression of $\alpha 5$ can protect carcinoma cells against apoptosis. These data suggest that growth suppression and anti-apoptosis may be $\alpha 5$ specific, and further indicate that there may be some intracellular proteins that interact with the $\alpha 5$ cytoplasmic tail to mediate these effects. To identify proteins that specifically interact with $\alpha 5$ cytoplasmic tail, the yeast two-hybrid system was used. We screened a mouse embryonic library fused with the VP16 activating domain, using the $\alpha 5$ cytoplasmic tail as "bait". From this screening, we identified a 500 bp partial sequence of a novel gene interacting specifically with the $\alpha 5$ cytoplasmic tail; this has been tentaviley named Integrin Binding Protein 1 (IBP1). Northern blot analysis revealed that the message of IBP1 is about 5.5 kB, and is highly expressed in brain and kidney. By screening the mouse brain cDNA library with the 500 bp fragment, we identified a 2.5 kB fragment of IBP1. Since this fragment did not have complete ORF, we screened the mouse library with a different probe and identified a fragment that has the rest of IBP1. Sequencing of this new fragment is in progress. Furthermore, we have made GST-fusion protein with 500 bp and 2.5 kB fragments of IBP1. Preliminary results using GST-fusion protein pull down experiments indicate that IBP1 interacts with human $\alpha 5$ integrin.

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EXPRESSION OF RECOMBINANT SOLUBLE $\alpha 4\beta 1$ INTEGRIN. ((K. Clark, P. Newham, S.E. Craig, J. Askari, M.J. Humphries.)) School of Biological Sciences, Manchester University, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT

The integrin $\alpha 4\beta 1$ is a receptor for both the extracellular matrix molecule fibronectin and the endothelial cell surface molecule vascular cell adhesion molecule-1 (VCAM-1). The expression profile of $\alpha 4\beta 1$ and that of its ligands implicates a major role in leukocyte extravasation at sites of inflammation. $\alpha 4\beta 1$ has been shown to have a role in several disease states including asthma, arthritis and atherosclerosis and thus is an attractive target for therapeutic intervention. To investigate the mechanism of integrin-ligand interaction and the effect of modulating agents, a soluble $\alpha 4\beta 1$ integrin has been engineered. The cDNAs for both the a and b subunits were altered by site-directed mutagenesis and constructs built for expression in COS-1 cells. Soluble integrin was isolated using affinity chromatography, but only a small amount of material was recovered from this transient expression system. A baculovirus expression system is currently being used to increase recombinant protein yields.

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TRANSMEMBRANE INTERACTION OF $\beta 1$ INTEGRIN WITH THE 16 KDA SUBUNIT OF VACUOLAR H^+-ATPASE ((M.A. Skinner, and A.G. Wildeman.)) Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Adhesive interactions between cells and the extracellular matrix play a central role in directing the migration, proliferation and differentiation of cells during growth and development. These interactions are mediated by integrins, a major family of cell surface receptors that bind components of the extracellular matrix, co-ordinate the actin cytoskeleton, and activate specific signal transduction pathways within the cell. We have identified a novel protein interaction between the $\beta 1$ integrin and the 16 kDa subunit of vacuolar H^+-ATPase (16K). This interaction was first isolated in a yeast two-hybrid screen and confirmed in an *in vitro* binding assay using bacterially expressed proteins. Immunofluorescent studies performed in L6 myoblasts expressing both native and epitope-tagged 16K demonstrate co-localization with $\beta 1$ integrin in focal adhesions. Deletion of the fourth of four transmembrane helices in 16K results in loss of interaction with $\beta 1$ integrin in both the two-hybrid system and in focal adhesions, suggesting that this helix is critical for the interaction. This helix is the one targetted by the human papillomavirus E5 oncoprotein upon its ligand-independent activation of the PDGF receptor. Fibronectin-coated latex beads used to cluster $\beta 1$ integrin also results in recruitment of 16K to the area of bead binding, providing evidence that ligand binding by $\beta 1$ integrin is important for the association of 16K and $\beta 1$. The data suggest that this interaction may have relevance to membrane signalling pathways involving integrins.

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EXPRESSION AND FUNCTION OF αv INTEGRINS DURING EARLY DEVELOPMENT OF *XENOPUS LAEVIS*. ((Benjamin G. Hoffstrom and Douglas W. DeSimone.)) Department of Cell Biology, University of Virginia, Charlottesville, VA. 22908

Integrin receptors are required for the assembly of extracellular matrices and cell migratory events that take place during amphibian gastrulation. In an effort to study the expression and function of αv integrins in these processes, we have produced two monoclonal antibodies (P3B6 and P3C12) that recognize *Xenopus* αv integrin receptors. A new *Xenopus laevis* cell line (S3-1) cultured from neurula stage dorsal explants was used to characterize antibody specificity and function blocking activity. Both antibodies immunoprecipitate a 150/90 kDa $\alpha v\beta$ integrin complex and stain tissue by immunohistochemistry. During embryogenesis, αv integrins are first detected in the oocyte and expressed at relatively constant levels through gastrulation with an increase in expression observed following neurulation. αv integrins are localized at the surface of all embryonic cells in blastula and gastrula stage embryos with asymmetries in the distribution only apparent at later stages. The two antibodies block S3-1 and embryonic cell attachment to vitronectin, but do not block attachment or spreading on fibronectin (FN). Blocking the function of αv integrins *in vivo* by antibody injection has no obvious effects on FN matrix assembly or gastrulation movements. Given that $\alpha 5\beta 1$ is the only other known FN receptor expressed prior to gastrulation, these results suggest that $\alpha 5\beta 1$ is likely to be the primary integrin involved in FN matrix assembly and integrin dependent adhesive interactions at gastrulation.

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THE EFFECT OF COBALT, TUNGSTEN, TUNGSTEN-COBALT, AND COBALT CHLORIDE ON L-SELECTIN EXPRESSION IN K562, J-11, AND DAUDI CELLS AND ON ICAM-1 EXPRESSION IN L2 CELLS. ((K.M. Starks and K.M.K. Rao)). Morehouse School of Medicine, Atlanta, GA and PPRB/HELD/NIOSH, Morgantown, WV 26505

We studied the effect of cobalt, tungsten and tungsten/cobalt particles on the expression of L-selectin at the protein level in a cell line (K562) transfected with a cDNA clone of L-selectin and in two lymphoid cell lines which express L-selectin constitutively (Daudi and J-11). Cell surface receptor expression was determined by flow cytometry. The cell lines were incubated with 5 µg/ml of the metal particles for 24, 48 and 72 hours. There was no difference in L-selectin expression in control and experimental samples. We also studied the effect of these metals on the expression of ICAM-1 in a rat lung epithelial-like cell line (L-2, ATCC) at the mRNA level. mRNA levels were measured by semi-quantitative RT-PCR. ICAM-1 mRNA was analyzed in L2 cells after 24h incubation with metal particles at 1 µg/ml, 3 µg/ml, 5 µg/ml, and 7 µg/ml. No significant differences were found in the ICAM-1 mRNA levels. In contrast, $CoCl_2$ at a concentration of 50 µM caused a significant decrease in ICAM-1 mRNA levels (40% of the control value; $P < 0.004$, $n = 4$). These data indicate that in short-term exposure experiments the hard metal particles have no significant effect on the expression of selectin and ICAM-1 family of adhesion molecules. The pathophysiology of hard metal disease remains to be elucidated.

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DEVELOPMENTAL REORGANIZATION OF $\alpha 1$ INTEGRIN. ((C. DiLullo, P.M. Mattioli and Christine McGinley)) Department of Anatomy, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131.

Integrins are known to be important in the developmental regulation of skeletal muscle. $\alpha 1$ integrin, identified as doublet bands in striated skeletal muscle, was investigated to ascertain any potential regulatory role for the protein in muscle development. Chick skeletal muscle was immunofluorescently labeled with antibodies to $\alpha 1$ integrin as well as to muscle specific proteins and examined at multiple developmental stages both *in vitro* and *in vivo*. $\alpha 1$ integrin was found to reorganize from a punctate distribution pattern into a periodic distribution pattern. In skeletal muscle cultures, non-striated, mononucleated myocytes exhibited $\alpha 1$ integrin staining in a diffuse punctate pattern. As the myocytes matured and under the appropriate conditions, $\alpha 1$ integrin began to reorganize into doublet bands that showed a sarcomeric periodicity. Both striated, mononucleated and multinucleated myotubes displayed the periodic $\alpha 1$ integrin pattern. The banded organization of $\alpha 1$ integrin can not serve as a scaffold for the assembly of striated myofibrils since striated myofibrils appear in the myofiber prior to $\alpha 1$ integrin periodic reorganization. In intact hindlimb myofibers, the reorganization of $\alpha 1$ integrin was analogous to that found *in vitro*. Day 14 myofibers exhibited a diffuse punctate distribution pattern with $\alpha 1$ integrin. By day 15, doublet bands were becoming apparent and by day 17, well defined $\alpha 1$ integrin doublet bands displaying a sarcomeric periodicity were detected.