SEA URCHIN (*STRONGYLOCENTROTUS DROEBACHIENSIS*) COELOMOCYTE SUBPOPULATIONS EXHIBIT DIFFERENTIAL EXTRACELLULAR MATRIX ADHERENCE AND INTEGRIN RECEPTOR LOCALIZATION

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Sea urchin coelomocytes consist of a number of different cell types, one of which, variously referred to as a phagocyte, bladder amoebocyte or simply coelomocyte, has been assigned roles in fluid clotting and phagocytosis. Our work and that of Edds has demonstrated that this particular class of coelomocyte actually consists of two distinct cell subtypes which differ in their relative degree of motility, overall morphology, and organization of their actin and tubulin cytoskeletons (Henson et al., 1992. *J. Cell Sci.* 103: 309-320; Edds, 1993. *J. Invert. Pathol.* 61: 173-178; Henson et al., 1993. *Bulletin MDIBL* 32: 4-6; Henson et al., 1993. *Mol. Biol. Cell* 4: 54a; Henson et al., 1994 *Bulletin MDIBL* 33: 9-10). The disc shaped stationary type 1 cells contain a sparse array of perinuclear microtubules and have actin filaments organized into an extensive cortical network which converges into radial perinuclear bundles. The polygonally shaped motile type 2 cells possess a well developed array of microtubules and have actin filaments arranged in parallel bundles similar to stress fibers. In the present study we have begun to elucidate the functional roles of these two coelomocyte subpopulations by studying their extracellular matrix (ECM) adhesion properties and their expression of integrin ECM receptors.

A mixed population of coelomocytes was collected from coelomic fluid in a calcium-chelating anticoagulant (0.5 M NaCl, 2.5 mM $MgCl_2$, 100 mM EGTA and 40 mM HEPES pH 7.4), isolated by means of centrifugation at 2,000xg onto a O.8 M sucrose cushion, and maintained in isotonic coelomocyte culture medium (CCM = 0.5 M NaCl, 2.5 mM $MgCl_2$, 1 mM EGTA and 20 mM HEPES pH 7.4). For cell adherence studies, mixed populations were allowed to settle onto either tissue culture-treated polystyrene plastic 6-well plates, or plates which had been coated with one of the following: 0.1 mg/ml poly-L-lysine (234,000 MW), rat tail collagen type I, human fibronectin, or Engelbreth-Holm-Swarm mouse tumor laminin (ECM coated dishes obtained from Becton Dickinson Labware, Bedford, MA). After 30 min the plates were rinsed extensively in CCM to remove nonadherent cells and the types of the remaining cells were scored by observing the plates with an inverted Nikon Diaphot microscope. Cell types were counted as a percent of the adherent population (see Figure 1). The presence of beta-integrin receptors in the cell types was tested for by immunoblotting and immunolocalization methods employing an affinity purified anti-sea urchin beta-integrin rabbit polyclonal antibody. For western blotting, lysates from cells were loaded onto SDS gels, transferred onto nitrocellulose and incubated in primary and alkaline phosphatase conjugated secondary antibodies. For immunolocalization of beta-integrin, cells were fixed in fresh 4% paraformaldehyde in CCM for 5 min followed by a 1 min post fix in -20°C methanol, blocked in PBS plus 1% BSA and 2% goat serum and then labeled with anti-beta-integrin antibody followed by the appropriate fluorophore conjugated secondary antibody. Labeled cells were viewed on a Nikon Optiphot II epifluorescent microscope using a Nikon 60X (N.A. 1.4) planapochromatic objective lens and photographed using Kodak TriX400 35 mm film.

Figure 1 shows the combined results of seven separate adhesion experiments. In general the cells adhered to polystyrene and poly-L-lysine surfaces in a percent ratio of 80%:20% type 1 to type 2 cells. This is the same percent ratio of cells that has been reported in the literature (Edds, 1993. *J. Invert. Pathol.* 61: 173-178). When settled onto collagen I the type 1 to type 2 percent ratio was approximately 45%:55% (Figure 1), although neither cell type appeared to spread well on this substrate. Preferential adherence of type 2 cells was observed on the laminin and fibronectin

substrates with the type 1 to type 2 cell percent ratio becoming skewed to approximately 10%:80% (Figure 1). On these substrates the type 2 cells were well spread and had large filopodial projections. Immunofluorescent localization of the beta-integrin subunit demonstrated that the plasma membrane of the type 2 cells stained intensely while type 1 cells were only weakly labeled (Figure 2). Western blotting indicated that the anti-beta integrin antibodies recognized an immunoreactive species in coelomocyte lysates at the appropriate molecular weight of 100 kDa.

The results of this study indicate that type 1 and 2 coelomocytes display differential ECM adherence properties as well as integrin receptor levels. The polygonal shaped and motile type 2 cells are shown to adhere better to the basal lamina components fibronectin and laminin and to stain heavily for the presence of the integrin receptors which link the cytoskeleton to the ECM. These characteristics of type 2 cells should allow them to bind to the basal lamina of endothelial layers during an extravasation process. In this way these cells could leave the coelomic fluid, pass through the endothelial layer and migrate into the tissues in a way analogous to how mammalian monocytes leave the blood and become migrating tissue macrophages. On the other hand, the type 1 cells appear to lack high levels of integrin and do not bind well to basal lamina components, and therefore would be more likely to remain in the coelomic fluid. This is consistent with these cells functioning as a platelet analogue involved in the process of coelomic fluid clotting. Future work will concentrate on quantifying the phagocytic potential of these two cell types, with the prediction being that the type 2 macrophage-like cells would be far more phagocytic than the type 1 cells.

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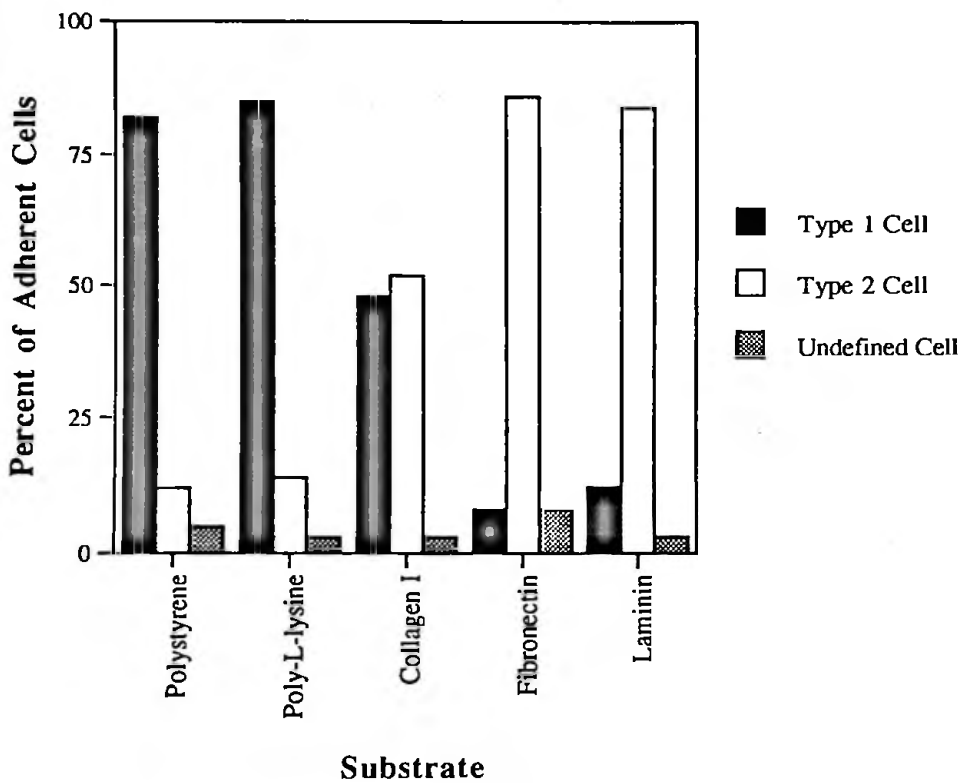

Figure 1: Results of seven separate cell adhesion experiments plotted as the means of the percent of adherent cells vs. substrate types for the three cell categories: Type 1, Type 2, and Undefined.

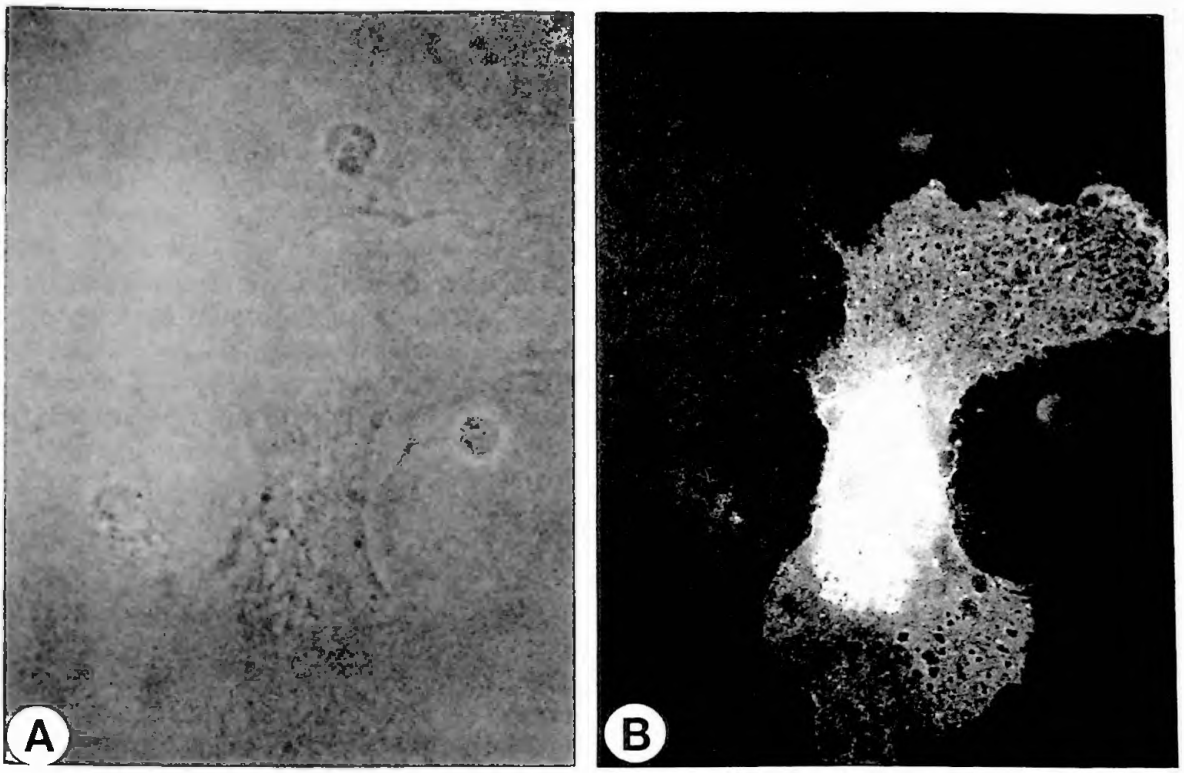

Figure 2: Phase contrast (A) and fluorescence (B) images of cells settled on poly-L-lysine and stained with anti-beta-integrin. The large polygonal shaped and heavily labeled cell in the middle is a type 2 while the three smaller discoid shaped and weakly labeled cells on the periphery are type 1. Magnification = 1,200 X.