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INVERTEBRATE DEFENSE SYSTEMS

Charles L. Greenblatt, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

It is generally agreed that acquired immune responses based on the γ globulin system are a late evolutionary development. However, Metchnikoff long ago showed that invertebrate forms do possess defense systems to cope with the intrusion of foreign infections and physical agents. Moreover, certain outstanding examples are known of symbiotic relationships of high specificity in the invertebrates. This project was an attempt to probe the mechanisms possessed by certain invertebrates for detection and sequestration of foreign substances.

The echinoderms were utilized since they possess a true coelom, are known to contain a rich variety of coelomic ("blood") cells, and, at least with respect to their eggs and sperm, possess determinant species-specific chemical groups. These studies have focused on the occurrence of accessory protein factors and the functional specialization of the various "blood cells" of the sea urchin, Strongylocentrotus droebachiensis.

The coelomic cells rapidly form a clot on removal from the organism. Boolootian and Giese (J. Exptl. Biol. 140:207-29, 1959) have shown that cysteine is an effective anticoagulant. We have extended these observations to other sulfhydryl compounds, using in the majority of our studies dithiothreitol (0.01 Molar final concentration). Since other redox compounds (DPNH, ascorbic acid, Janus Green B, 2,6-dichloroindophenol) were not effective and sulfhydryl inhibitors (mersalyl, alloxan, and iodoacetamide) were also inactive as anticoagulants, it is unlikely that the role of the anticoagulant is to provide a reducing environment and especially one which maintains -SH groups. Calcium removal and replacement as well as chelating compounds did affect the cellular adhesion of the coelomic cells, but they did not seem to control gross clot contraction. Materials were fixed for electron microscopy, and these micrographs should aid in discriminating some of the cellular interactions in clot formation. Large amounts of intercellular fibrin-like protein do not seem to be important, although some small amount of protein is there and we are presently attempting to characterize it.

Both separated and clotted cells were challenged with polystyrene spheres and zymosan granules. In general the cells within clots seemed more active in phagocytosis than isolated ones, as though clotting activates phagocytosis of exogenous particles. Although mammalian macrophages require accessory proteins in order to be able to ingest zymosan granules, the sea urchin cells were able to take them up without the assistance of such factors. High levels of acid phosphatase are measurable in these cells, and we are hopeful of localizing the enzyme by appropriate cytochemistry.

Finally, one group of cells contains napthoquinone pigments. Dr. W. Kinter has helped us in characterizing the in vivo spectra of these cells under a variety of conditions. We are attempting to correlate these data with a series of spectra of the isolated pigment at different hydrogen concentrations and with various chemical agents.