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Meeting Report

Of Umbrellas, Jazz and Slow-steppin'

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Workshop on Apoptotic Cell Recognition, El Escorial Monastery, Madrid, Spain, 9–10 May 2000

As any mystery reader knows, getting away with murder is much easier when there is an efficient way to get rid of the corpse. Nature knows the same rule, and the much-studied process of programmed cell death, or apoptosis, includes a means for disposing of the dying cell by phagocytosis. The past year has seen several interesting new developments in the study of this terminal stage of apoptosis, and these were among the subjects of a rare meeting devoted solely to this subfield, a workshop held at the Escorial near Madrid on May 9th and 10th following the general meeting on apoptosis sponsored by the Cell Death Society. Appropriately enough, the opening talk, by Michael Hengartner (Cold Spring Harbor), reported that in the presence of a ced7 engulfment mutation a weak ced-3 (caspase) allele resulted in the appearance of cells which refused to complete the apoptotic sequence, or even retreated towards a normal morphology, suggesting that in *C. elegans* the dying and engulfing cells collaborate to bring apoptosis to a final conclusion.

The first of the two sessions concentrated on the signaling pathways and machinery of the engulfing cell. Proteins identified in genetic screens as required for engulfment of apoptotic cells in *C. elegans* are now being confirmed to play a similar role in other organisms. E Smits (Ghent) showed that over-expression of the human ortholog of one such gene (*cde6*) promoted phagocytosis of apoptotic cells. In the past year, another group of worm engulfment genes have been identified as elements in a crk/dock180/rac signaling pathway (Reddien and Horvitz, *Nature Cell Biol*, **2:** 131 (2000). In the general meeting, Raymond Birge (Rockefeller) set the stage for the workshop sessions with the suggestion that this pathway was activated in mammalian dendritic cells by binding of apoptotic cells to the $\alpha_V\beta_5$ integrin receptor. Hengartner continued the theme by describing experiments placing the product of the *ced-12* gene in this same pathway. A *Drosophila* homolog of the dock180 protein is the product of the *myoblast city* gene, and Susan Abmayr (Penn State) gave a description of the defects in these mutant flies, which include deficiencies in ectodermal migration with variable penetrance, and a block to myoblast fusion with uniformly complete penetrance, but with no known effect of any allele on removal of apoptotic cells, and particularly the unfused myoblasts. Recent progress on the isolation of the *Drosophila* gene *crkII* strengthens the value of this organism for analysis of this conserved signaling pathway. Philippe Chavrier (Marseille) discussed the role of related pathways involving rac1 and cdc42 in Fc receptor-mediated macrophage phagocytosis, and particularly in pseudopod extension and membrane fusion to form the phagosome. Given the general features of these pathways, the implication seems to be that removal of apoptotic cells may require activation of a particular and perhaps dedicated machinery required by phagocytes for movement and physical engulfment.

The alternative approach to genetic screens in identifying proteins required for phagocytosis of apoptotic cells has been the use of antibodies and other agents which inhibit uptake of apoptotic cells. These two approaches have converged in the identification of receptors expressed on *Drosophila* phagocytes (hemocytes). Natalie Franc (Mass General) summarized the defects of mutants in the *Drosophila* gene *croquemort*, which codes for a scavenger receptor. Extending the genetic screens to uncover mutations blocking removal of apoptotic cells identified genes which block hemocyte formation, emphasizing the importance of this class of cells in removal of apoptotic cells in flies. Chris Gregory (Nottingham) summarized progress on studies of two mammalian surface proteins, CD14 expressed on phagocytes and ICAM3 present on apoptotic lymphocytes, identified by antibody inhibition as involved in the phagocytic process. The latter is important because it is the only clearly defined ligand identified on the apoptotic cell surface. The possibility that one of the proteins serves as the counter receptor of the other was advanced, and initial studies with tissues from CD14 knockout mice were presented.

One of the hallmarks of the recognition of apoptotic cells is the disruption of the normal asymmetric distribution of phospholipids across the plasma membrane, and the exposure of phospholipid, phosphatidylserine (PS), on the apoptotic cell surface. In the second session, Philippe Devaux (Paris) summarized succinctly the contentious state of knowledge of the enzymes which transport phospholipids from one side of the membrane to the other, governing lipid asymmetry, and B Gleiss (Karolinska) presented evidence that some cell lines fail to alter these transport mechanisms during induced apoptosis, maintaining asymmetry. The significance of these findings was emphasized by Robert Schlegel (Penn State), who argued that loss of lipid asymmetry is the key step which activates the several receptors and ligands, on both the target and phagocyte surface, implicated in recognition and engulfments of apoptotic cells. One of the proteins involved in these processes in *C. elegans* is an ABC transporter, the product

of the *ced-7* gene. G Chimini (Marseille) summarized what is currently known of the roles played by the human homolog of the gene, *ABC1*. Analyses of mice in which the gene has been knocked out suggest that the defect simultaneously impairs removal of apoptotic corpses, for example in the developing limb bud, alters the phospholipid scramblase activity required to expose PS on the cell surface, and blocks cholesterol efflux from cholesterol-loaded cells. The latter phenotype, known as Tangier disease when the gene is mutated in humans, links the phagocytosis process to cholesterol metabolism (as does the involvement of scavenger receptors such as CD36). Understanding why these two processes are linked will be an exciting problem to solve.

The mechanism by which PS on apoptotic cells is recognized by other cells was the subject of a talk by Valerie Fadok (Denver), who described a protein which, when expressed on the cell surface, renders uptake of apoptotic cells sensitive to inhibition by PS vesicles. Antibodies to the protein, like apoptotic cells and PS vesicles, promote anti-inflammatory responses (TGFβ production, and a block to TNFα secretion). Dvor Mevorach (Tel Aviv) also emphasized that PS exposure on apoptotic cells recruits the complement protein C3bi, which enhances integrin-mediated phagocytosis of apoptotic cells. The PS-specific probe, annexin V, is now commonly used to monitor PS exposure on apoptotic cells. Chris Reutelingsberger (Maastricht) described the extension of this technique into the clinic, where 99 mTc-labeled annexin V and single emission computerized tomography have been used to visualize PS-exposing cells in the hearts of patients with acute myocardial infarcts.

Overall, the workshop reflected the considerable progress which has been made, using the converging, separate threads of genetic *vs* biochemical analysis, to identify (a) the receptors and ligands, both protein and lipid, and (b) the signaling pathways and machinery required for recognition and engulfment of apoptotic cells. In the process, however, as is inevitably the case, there are new questions, such as how transbilayer lipid rearrangements alter protein behavior, why such rearrangements are necessary on both target and phagocyte, how the process is linked to cholesterol metabolism, and how these developments will change our view of diseases and their diagnosis. These questions promise to keep the area of corpse removal as lively as a New Orleans funeral parade.