STUDY OF APOPTOTIC CELL DEATH BY ANTIBODY PROBES IN CELL CULTURE

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Apoptosis is a central event in biology. Programmed cellular suicide is intimately involved in organ sculpting during development, and also plays a crucial role in the homeostasis of adult tissues. Growth factor withdrawal, viral or chemical transformation, or exposure to high-energy radiation can all trigger cell death cascades, as can the action of T cells upon infected targets. As cell death pathways have evolved, so have mechanisms for the efficient recognition and removal of cellular debris.

Aberration in either the mechanisms of apoptosis or in the mechanisms of dead cell removal can have profound physiological consequences. Many pathologies of unknown etiology, such as Alzheimer's Disease and Systemic Lupus Erythematosus (which otherwise may share little in terms of clinical manifestation) have been linked to dysfunctional apoptosis. Interestingly, both these disease are associated with cognitive dysfunction.

Apoptosis is characterized by the extrusion of "blebs", membrane-bound vesicles that contain packaged cellular material. Many of the moieties contained in such blebs are targets of autoimmune responses in Systemic Lupus Erythematosus (SLE), and are thought to be the original antigenic insult for the initiation of disease in genetically susceptible individuals. A significant autoimmune target is DNA; antibodies to double stranded DNA show close association with nephritis in established disease. Another interesting correlate is that of antibodies to ribonucleoproteins (also present in the blebs), and the neuropsychiatric symptoms observed in SLE. Associated with multi-organ pathology, other membrane-associated antigens are also targeted. For example, antibodies that bind to, and induce the death of, healthy cells also arise. Non-cytotoxic antibodies that induce more subtle, anti-proliferative effects may mediate immune dysfunction. In many instances, the fine specificities of all these antibodies are unknown.

This study sought to investigate these phenomena by generating human monoclonal

antibody probes, either reactive against healthy cells, or preferentially targeting apoptotic cells.

Several hundred lymphoblastoid cell lines were established from peripheral blood B cells; screening procedures determined the presence of antibodies specifically directed against either healthy or apoptotic cells. Cells secreting antibodies of interest were fused with appropriate myeloma partners. Human monoclonal antibodies were generated after standard sub-cloning procedures and characterized for antigenic reactivity, biological function and variable region sequence.

An antibody (PR5) reactive against the cell surface was generated; Fluorescence Activated Cell Scanning (FACS) results indicated broad cellular specificity. The antibody had several biological effects; it induced the complement-mediated killing of a variety of cell types, and exhibited anti-proliferative effects in several different assays. Of particular interest was the reactivity of the antibody against human neuronal and glial cells. Screening a human brain cDNA library using PR5 as a probe identified the post-synaptic protein CRIPT as a target antigen. This protein has previously been known to induce the redistribution of PSD-95 to microtubules (via interaction with b -tubulin), thus helping to bridge it to the cytoskeleton; PSD-95 is itself a scaffolding protein and is associated with other membrane proteins and signaling molecules. Analysis of the PR5 light chain showed it to be a member of the Vk III family, with the closest germline gene being L6; the Jk 2 segment was employed. The heavy chain showed belonged to VH1 family and had maximum homology to the 08 germline sequence; D1-26 and JH4a were employed, with several deletions and modifications, in addition to extensive junctional diversity.

Another antibody (RN86) was also generated, which was non-reactive towards healthy peripheral cells while specifically recognizing cells undergoing apoptosis; after apoptotic stimuli, antibody reactivity was observed subsequent to Annexin-V reactivity (indicating recognition of cells at a relatively late stage of the death cascade), but before cells became permeable to vital dye. The antibody was shown to recognize cytoplasmic antigens that translocated to the cell surface during the process of cell death. Interestingly, RN86 also recognized cell surface moieties present on healthy, non-apoptotic glial and neuronal cells. Enzyme-linked immunosorbant assays (ELISAs) on a panel of autoantigens consistently targeted in SLE provided details as to the fine specificity of RN86; Ro60 and Ro52 were recognized. These molecules (of molecular weight 60 KDa and 52 KDa respectively) are ribonucleoproteins of unknown function. The RN86 light chain belonged to the Vk I family (with 12 being the closest germline gene) and the Jk 1 segment was employed. The heavy chain belonged to the VH3 family with 53 being the closest germline gene; the D3-10 and JH6 gene segments were employed, incorporating base deletions and additions. In comparison to the PR5 heavy chain however, fewer amino acids mutations were seen.

The natural fate of apoptotic cells *in vivo* is phagocytosis. The influence of the apoptotic cell-specific antibody RN86 upon this process was therefore ascertained; the antibody had a significant inhibitory influence upon phagocytic uptake of apoptotic cells, whereas PR5 antibody showed no such effects. RN86-opsonized apoptotic cells elicited significantly lower TGF-b secretion, whereas as PR5-opsonized apoptotic cells

significantly enhanced TNF-a secretion, both potentially pro-inflammatory events.

Anti-idiotypic responses to RN86 were investigated. Since one of the recognized proteins (Ro60) was known to exist *in vivo* in association with another protein La (not recognized by the antibody), it was postulated that the anti-idiotypic response would be characterized by an "antigenic spread" to La. Characterization of purified anti-idiotypes revealed more extensive reactivity than anticipated. Compared with RN86, grossly distinct reactivity was observed upon Western blots; both healthy cell- as well as apoptotic cell- surface reactive components of the immune response could be discerned; cytoplasmic reactivity was also apparent in immunoflourescence assays. As postulated, anti-La reactivity was observed; several other tested autoantigens were also targeted, and many others probably remain un-characterized. Quite unexpectedly, the anti-idiotypic response to an apoptotic cell specific human monoclonal antibody therefore diversifies to include many additional molecules that exist upon the cell surface of healthy as well as apoptotic cells, and in the cytoplasm.

This work has investigated the possible physiological consequences of human autoimmune antibodies directed towards plasma membrane moieties on either healthy or apoptotic cells. It is apparent that such humoral responses, common in diseases like SLE, can have significant down-stream effects, many of which may be capable of influencing processes both within and outside the immune system.