

Master Answers to Clinical Immunologic Assays-II

Comments on immunofluorescence (IF)

1. IF is commonly used by pathologists to confirm the presence of antibodies bound in renal glomeruli and skin. There is a wide range of commercially available reagents that can be used to localize immunoglobulins of all isotypes, complement components, fibrin, etc. in biopsy or autopsy tissue. In fact, if antibodies can be made to something, then a researcher can set up an IF assay that will detect the antibody bound to that something (antigen) in tissue. Direct fluorescence is when a specific fluorescinated antiserum to an antigen is used to probe tissue for the antigen. Direct fluorescence is used in the diagnosis of many infectious diseases. Indirect immunofluorescence is the use of a fluorescinated anti-antibody to a human immunoglobulin that the clinician suspects is causing pathology. This is a common procedure and used routinely when studying renal biopsies. Understanding how to interpret IF will become evident in a later Small Group

Questions on flow cytometry:

- 1. The beauty of flow analysis is that the clinician can customize a search for specific CD (or other) markers by developing monoclonal antibodies specific for surface markers that will provide critical clinical information. Leukemias and lympho-proliferative disorders were the first logical targets for monoclonal probes because of the information already available on their characteristic CD markers. For example, if the patient has clinical characteristics of a T cell leukemia, CD3 might be one of the markers used. In the case described, all we know is that the white blood cell count is markedly elevated. Using markers for granulocytes, lymphocytes and monocytes would determine the lineage of the cell. Once the lineage is determined, you would then choose the markers that would detect specific phenotypes. In this case, it might be that lymphocytes were the expanded cell population. You would then select CD3 and B cell markers that could sort out the 2 populations. Then, if the expanded population was B cell in origin, you could easily use kappa and lambda chain markers to determine clonality. If there is a skewed distribution of kappa or lambda chains in the cell population, the likely assumption is that there has been proliferation of a single clone of B cells. The more precise the diagnosis, the more targeted the therapy and prognosis will be.*
- 2. Clonality of immunoglobulins can be determined by a simple 2 step process. First separate serum proteins out by subjecting them to a polarized electrical field. The Igs will migrate to the gamma region, albumin will go the other way. If a patient has been undergoing intense antigen stimulation from an infection, numerous clones of plasma cells will be making antibodies to the infecting organism and each plasma cell clone antibody will migrate to a unique spot in the electrical field because each clone is producing a slightly different antibody in response to the infection. The result will be increased total amount of antibodies that are spread across the entire gamma region. The converse will be true if one clone of plasma cells, usually malignant, is producing*

large amounts of identical antibody with identical size, charge and shape. These molecules will migrate to one site in an electrical field and look like a spike.