Laboratory Diagnosis of Immunologic Disorders

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Preface

Rapid progress in immunology during the past two decades has led to the widespread application of basic advances to clinical medicine. As a result, immunologic methods of laboratory diagnosis have been increasingly, but often uncritically, used in internal medicine, pediatrics, rheumatology, hematology, allergy, and oncology. This symposium was aimed at relating the current knowledge of the diagnostic methods in humoral and cellular immunity, transfusion and transplantation, immediate and delayed hypersensitivity, and immunodiagnosis of cancer to the daily practice of laboratory medicine.

Laboratory medicine attempts to meet the multiple challenges of improving availability, precision, and accuracy of measurements of known medical importance; to assure their proper interpretations; to introduce new tests; and to assess their significance and utility in patient management. This last task has become particularly onerous in immunology. Numerous new tests are now available. Some of them are biologic assays that cannot yet be reduced to exact chemical measurements. Different laboratories become experts in one or the other approach. In the welter of possible choices, the nonexpert is usually left to either follow the most recent, as yet unconfirmed, publication or his own anecdotal experience.

To assist both the laboratory scientists and the clinicians in the selection of tests and their interpretation, the present symposium has brought together experts on virtually every immunologic disorder. The eminence of the faculty was matched by their willingness to go beyond their areas of personal research interests and to review what is available in the laboratory and useful in patient care. By design, the result is not a laboratory manual, but a comprehensive guide to the evaluation of the few old and the many new tests in the field.

We trust the readers will share our view that the assigned task has been accomplished by the contributors, who have our sincere gratitude. The success of the symposium was due in large part to the hard work of the staff of the University of California Continuing Education in Health Sciences, particularly Renee Vandergrift and Sadie Kaye. The editorial assistance of Patricia Diridoni and Warren Eveleth greatly aided us in compiling the proceedings of this symposium.

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SECTION I

Immunochemistry

Moderated by Girish N. Vyas

Edward C. Franklin

1

Electrophoresis and Immunoelectrophoresis in the Diagnosis of Dysproteinemias

There are few if any cells in the body that lend themselves as readily to precise biochemical analyses as the plasma cells and lymphocytes. This favored position is largely due to the facts that each clone of cells secretes a single homogeneous protein that often constitutes more than 20% of its total synthetic product, and that the type of protein produced generally remains constant for many generations. Because there exist more than 40 known classes and subclasses of heavy (H) chains, at least eight subclasses of light (L) chains, and many thousands of H and L chains differing in their primary structure, it will ultimately be possible to identify and follow the fate of virtually every clone biochemically by examining the secreted immunoglobulins.[1] The normal immunoglobulin fraction consists of a large number of classes and subclasses of immunoglobulins and thousands of different antibody molecules, and there is a general increase in many of them in diseases associated with a diffuse state of hypergammaglobulinemia, such as is often encountered in chronic infections, cirrhosis of the liver, systemic lupus erythematosus, (SLE), rheumatoid arthritis, and a host of other diseases. Thus, electrophoresis and immunoelectrophoresis, and also immunoglobulin quantitation, do not generally detect specific changes in immunoglobulins and are consequently generally of little value diagnostically in these instances. In contrast, these techniques are very useful in those diseases where a single clone of plasma cells or lymphocytes proliferates to produce large amounts of a homogeneous immunoglobulin or immunoglobulin fragment that is readily detectable as a narrow band on ordinary serum electrophoresis. Unfortunately, the concentration, mobility, or appearance of such an electrophoretic spike on

paper or cellulose acetate electrophoresis rarely if ever permits a more precise classification of the type of protein. The appearance and mobility of homogeneous spikes are not class- or subclass-specific. Therefore, it is generally not possible to distinguish one type of proteins from others, with the possible exception of the broad spike often seen in IgA myelomas and some of the abnormal proteins seen in gamma heavy-chain diseases. Hence, it is necessary to employ other techniques, especially those relying on the antigenic features of these molecules, if one wishes more precise characterization of the proteins produced.[2,3]

In ordinary clinical use, it is obviously not possible to study these molecules biochemically in terms of amino acid sequence. The amount of work involved would be far in excess of the possible benefits to be derived. During the past 20 years, we have learned to take advantage of the fact that the amino acid sequence differences are reflected in the antigenic properties of the molecules. By using homogeneous proteins belonging to the various classes and subclasses as antigens, and by making the antisera specific by simple absorption with fractions devoid of the protein in question, it is possible to make antisera specific for each of the known immunoglobulin classes and subclasses of the constant regions of the H and L chains and, in the case of the L chains, also for the variable region subclasses.[4] To date it has not been possible to make similar antisera specific for variable heavy-chain regions. In certain instances, the differences between them are so subtle that it is necessary to produce antisera in primates or animals rendered tolerant to the common antigenic determinants of immunoglobulins. In general, the antisera are rendered specific by absorption with cord sera (devoid of IgM and IgA); agammaglobulinemic sera, virtually devoid of all immunoglobulins; or purified myeloma proteins, macroglobulins, or kappa or lambda Bence Jones proteins of a different type than the antigen used for immunization.

SPECIFICITY OF ANTISERA

For most purposes in clinical practice, use of antisera specific for the five classes of H chains, the four subclasses of gamma and two of alpha chains, and kappa and lambda light chains is sufficient to permit classification of homogeneous proteins. It should be remembered, however, that every myeloma protein differs from almost every other myeloma protein, and that these differences can be recognized in their so-called "idiotypic" antigenic determinants. As a result, it is theoretically possible to make specific antibodies to virtually every myeloma protein and, ultimately, to recognize and classify every protein produced in disease. Based on experiments with murine

plasma cell tumors and certain selected human antibodies, these idiotypic antisera may be directed to the antibody-binding site and hence react with all molecules having the same antibody specificity.[5]

It is obvious that such precise classification is of little general value in clinical practice, but it is of great value in increasing our understanding of antibody structure. To accurately classify the type of plasma cell or lymphocyte neoplasm, a goal that can generally not be achieved either on the basis of the clinical or pathologic features, it is generally sufficient to determine only the H and L chain classes. If reagents are available, determination of the H chain subclass may also be of great clinical value because, with increasing experience, it has become apparent that certain features, such as hyperviscosity and cryoglobulinemia, are more frequent with certain subclasses of IgG than with others and that amyloidosis is most often associated with V-lambda-1 light chains. It seems likely that other clinical correlations may be noted as a result of careful immunologic classifications in the future.[1]

USEFUL DIAGNOSTIC TESTS

A detailed biochemical and immunologic approach to the classification of plasma cell and lymphocyte neoplasms is of value for the following reasons: (1) It allows precise classification of the type of disorder, which is not always possible on clinical or pathologic grounds. In certain instances, such as macroglobulinemia, heavy-chain diseases, or L-chain production, this classification correlates with the clinical features of the disease and, on occasion, also with the prognosis. In many instances, the type of protein produced and the amount present may influence the type of therapy to be used, i.e., chemotherapy, plasmapheresis, etc. (2) It permits clear-cut insights into the biosynthetic processes occurring in normal and also in pathologic neoplastic cells and hence significantly increases our understanding of factors controlling immunoglobulin synthesis. (3) It may ultimately lead to clinical and biochemical correlations that we are not yet aware of on the basis of our still-limited biochemical analyses. (4) Careful characterization and detailed studies of the products of these cells have provided clear insights into the normal biochemistry and physiology of plasma cells and lymphocytes and in the last 10 years have been directly responsible for virtually all the important advances in immunochemistry that have taken place.

Great progress has been made in the last 30 years in the tools available to the clinician in achieving these goals. Initially, only free electrophoresis combined with ultracentrifugation was available. This allowed the differentiation of a 7S from a 19S immunoglobulin. Each of these tests was complicated and time-consuming and not practical for routine clinical use. The in-

troduction of electrophoresis on paper or other solid supporting medium such as cellulose acetate, made the detection of homogeneous proteins easier, but did not aid significantly in their precise identification. Only with the introduction of immunoelectrophoresis with specific antisera for classes and subclasses of immunoglobulins has it been possible to develop simple tests that can be applied in every clinical laboratory, requiring only the availability of specific antisera to permit the necessary typing and classification of immunoglobulins. As a result of the widespread use of this technique, the recognition of diseases with homogeneous proteins has significantly increased, and several new entities, such as the group of heavy-chain diseases, have been discovered.[6] In addition, paper or cellulose acetate electrophoresis, combined with initial characterization of the type of protein by immunoelectrophoresis, is generally as effective in quantitating the amount of an immunoglobulin fraction as is immunoglobulin quantitation. Hence, this combination is of great value in following the course of a patient or his response to therapy because in most patients more than 90% of the protein associated with the spike consists of the homogeneous component in question.

CLINICAL APPLICATIONS

Let us first consider the work-up of an individual suspected of having a plasma cell or lymphocyte neoplasm or inadvertently found to have a homogeneous protein spike on electrophoresis. We shall limit ourselves to discussion of individuals whose sera contain homogeneous immunoglobulins, polypeptide chains, or fragments. The best screening test is paper or cellulose acetate electrophoresis. If a homogeneous band is found, one generally cannot classify it precisely, and immunoelectrophoresis with antisera for heavy and light chains is the next logical step. If the initial electrophoretic analysis is negative, it is unlikely to find significant amounts of a homogeneous protein on immunoelectrophoresis with the exception of certain patients with gamma, alpha, and mu heavy-chain diseases or some individuals with L chains in the serum. Because of the not infrequent occurrence of these entities and the great interest in discovering them, it is now generally felt that if the clinical state warrants it, even a negative electrophoretic serum analysis should not deter one from a further search for an abnormal protein by immunoelectrophoresis.

Let us look next at some practical illustrations of the use of immunoelectrophoresis in clinical medicine. Figures 1-1–1-3 summarize the major types of patterns one encounters in clinical practice. In the most common type of disorder, namely production of an intact immunoglobulin, the pattern will show a prominent precipitin arc whose appearance is the same when