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Summary of training in laboratory diagnosis of autoimmune diseases

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The prevalence of various autoimmune diseases in the general population is on a steady increase, therefore great efforts have been invested in the field of serodiagnosis for the earliest possible diagnosis, use of appropriate therapy, and monitoring of its efficacy. These endeavors result in virtually daily discovery of ever newer autoantibodies, which have been introduced in the algorithms for laboratory diagnosis of particular autoimmune diseases because of their high sensitivity and specificity. As I work at Department of Laboratory Immunology, it was of utmost importance for me to have an opportunity to visit a renowned laboratory engaged in the serodiagnosis of autoimmune diseases and to be introduced in the structure of such a laboratory, the methods employed, work-up guidelines for patients suspect to suffer from an autoimmune disease as well as for the highly valuable exchange of experience. Owing to the IFCC support, I had an opportunity to visit Department of Clinical Chemistry and Molecular Diagnosis, Central Laboratory, Giessen and Marburg University Hospital in Germany. The education curriculum was supervised by Professor Harald Renz, head of Department, and Ileana Herzum, MD, his coworker.

The curriculum included mastering the method of indirect immunofluorescence (IIF) on various substrates, enzyme-linked immunosorbent assay (ELISA) and immunoblot (IB) method; use of algorithms for particular antibody classes; and correct interpretation of the finding obtained in the context of the referral diagnosis.

The basic principle of all methods employed in the serodiagnosis of autoimmune diseases is detection of the immunocomplex formed by binding of the sample antibody, target antigen and anti-human immunoglobulin labeled with fluorescein in case of IIF method and with enzyme in case of ELISA and IB methods. In IIF method, the antigen is found in its native form on the fixed cryostatic tissue sections or cells from the culture. In ELISA, the antigen from tissue extracts or recombinant antigens are bound in wells, whereas in IB method the antigens from tissue extracts are separated by SDS gel electrophoresis according to their molecular weight and then transferred to nitrocellulose strips, or recombinant antigens are directly applied onto the strips.

The identification of antinuclear antibodies (ANA) is the key parameter in the laboratory diagnosis of systemic autoimmune diseases: Systemic lupus erythematosus and its variants, primary Sjögren syndrome, the various types of systemic sclerosis and idiopathic myosistis (poly- and dermatomyositis). The detection of certain ANA allows the classification of symptoms with manifold differential diagnostic implications and in some cases has a prognostic significance. According to the algorithm for ANA detection, the first step includes screening by use of IIF method on Hep-2 cells (human epithelioma type 2 cells from cell culture) at the basic dilution of 1:80. The samples with positive fluorescence are set again at increasing double dilutions to determine antibody titer, and final dilution yielding positive fluorescence. A titer of ≥1:160 is considered clinically relevant. Homogeneous fluorescence on Hep-2 cell screening test points to additional sample testing for the presence of antibodies to double strand DNA (dsDNA) by IIF method on the Crithidia luciliae monoflagellate, and for the presence of antibodies to histones by ELISA. If the fluorescence on Hep-2 cell screening test is granular, detection of ENA (extractable nuclear antigen) antibodies follows, i.e. SS-A (Ro), SS-B (La), Sm, and U1-RNP, by semiquantitative ELISA. When the titer of antibodies yielding homogeneous fluorescence is >1:640, detection of ENA antibodies is also performed because intensive homogeneous fluorescence may mask granular fluorescence. The remaining types of fluorescence on Hep-2 cells do not require additional testing, except for the characteristic nucleolar fluorescence that points to Scl-70 antibodies, or cytoplasmic fluorescence characteristic of Jo-1 antibodies or antibodies against ribosomal P protein, the specificity of which, likewise Scl-70, is confirmed by semiquantitative ELISA.

Antineutrophil cytoplasmic antibodies (ANCA) are an important serologic marker of systemic small vessel vasculitides. IIF method on ethanol fixed granulocytes is used as a screening test for ANCA detection, and on formaldehyde fixed granulocytes for pANCA differentiation from ANA. According to the algorithm, the finding of positive cytoplasmic (cANCA) or perinuclear (pANCA) fluorescence on ethanol/formaldehyde fixed granulocytes is followed by determination of antibody titer, while specificity is confirmed by semiquantitative ELISA allowing simultaneous detection of 6 antibodies to relevant granulocyte antigens: proteinase 3, myeloperoxidase, lactoferrin, elastase, catepsin G, and bacterial permeability increasing protein (BPI).

Serodiagnosis of autoimmune diseases of the liver includes detection of antibodies against hepatic antigens: antimitochondrial antibodies (AMA), smooth muscle antibodies (SMA), liver/ kidney microsomal antibodies (LKM), antibodies against liver cell membrane (anti-LMA) and liver-specific protein (LSP). IIF on combined substrates, i.e. cryostatic sections of rat stomach/rat kidney/primate liver/rat liver, is employed as a screening method, allowing for simultaneous detection of all antibodies listed above. Depending on the antibody detected, titer is determined on standard substrates, i.e. on rat kidney for AMA antibodies, on rat stomach for SMA antibodies, and on rat liver for LKM antibodies. According to algorithm for the detection of AMA, a positive finding of IIF testing on the rat kidney and stomach cryostatic sections is followed by the antibody titer determination, whereas specificity for M2 mitochondrial target antigen as a highly specific diagnostic marker of primary biliary cirrhosis is demonstrated by IB method. A positive finding of LKM antibodies on the rat liver and kidney cryostatic sections is also followed by determination of titer and specificity for LKM-1 antigen (positive in

autoimmune hepatitis type II) by IB method.

The algorithm for the diagnosis of celiac disease includes determination of antiendomysial antibodies (EMA-IgA) by IIF method on cryostatic sections of monkey intestine and antigliadin class IgA antibodies (AGA-IgA) by ELISA.

IIF method on two standard substrates, primate cerebellum and peripheral nerves, is used on detection of anti-neuronal antibodies: antibodies to Purkinje cell cytoplasm (anti-Yo), antibodies to neuronal nucleus (anti-Ri and anti-Hu) associated with paraneoplastic syndrome, and antibodies to myelin the role of which in the diagnosis of multiple sclerosis remains controversial. The simultaneous use of primate intestine as an additional substrate enables reliable differentiation from other antibodies (ANA), i.e. distinguishing anti-Hu antibodies reacting with cell nuclei of plexus myentericus in the intestine from anti-Ri antibodies.

In addition to the diagnosis of autoimmune diseases, I also had an opportunity to observe the use of a novel method in monitoring patients with sepsis, developed by Becton Dickinson on a FACScan flow cytometer. The method is based on quantitative determination of individual expression of HLA-DR molecules on CD14 positive cells. Reduced expression of HLA-DR molecules is characteristic of functional monocyte deactivation, thus being considered a good parameter of impaired immunocompetence. Test kit contains anti-HLA-DR antibody that recognizes Q MHC II antigen and antibodies recognizing CD14 and CD64 antigens on monocytes. HLA-DR expression is defined as the mean number of HLA-DR molecules on the surface of one monocyte (mAb/cell).

To conclude this report, I have to emphasize the high professional value of my training visit to the laboratory of Marburg University Hospital, i.e. the opportunity to master the methods of detection of various antibodies, including some very rare ones, and to collaborate with the highly competent staff with rich experience in the diagnosis of autoimmune diseases.