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Review

# Cellular analyses in the monitoring of autoimmune diseases

Ulrich Sack [a,*], Andreas Boldt [a], Nora Mallouk [b], Rudolf Gruber [c], Veit Krenn [d], Anne-Emmanuelle Berger-Depincé [e], Karsten Conrad [f], Attila Tarnok [a], Claude Lambert [g], Dirk Reinhold [h], Stephan Fricke [i]

[a] Universitätsklinikum Leipzig, Department für Diagnostik, Institut für Klinische Immunologie, Johannisallee 30, 04103, Leipzig, Germany
[b] URCIP, CHU Saint-Etienne, Hôpital Nord, 42055 Saint-Etienne Cedex 02, France
[c] Institut für Labormedizin, Mikrobiologie und Krankenhaushygiene, Krankenhaus Barmherzige Brüder Regensburg, Prüfeninger Straße 86, 93049, Regensburg, Germany
[d] Medizinisches Versorgungszentrum für Histologie, Zytologie und Molekulare Diagnostik Trier, Max-Planck-Str. 5, 54296, Trier, Germany
[e] Laboratoire d'immunologie Clinique CHU Saint-Etienne, Hôpital Nord, 42055 Saint-Etienne Cedex 02, France
[f] Institut für Immunologie, Medizinische Fakultät "Carl Gustav Carus" der Technischen Universität Dresden, Fetscherstraße 74, 01307, Dresden, Germany
[g] Immunology laboratory, Pole de Biologie-Pathologie, University Hospital. CNRS UMR5307 Labo Georges Friedel (LGF); 42055 Saint-Etienne Cedex 02, France
[h] Otto-von-Guericke-Universität Magdeburg, Medizinische Fakultät, Institut für Molekulare und Klinische Immunologie, Leipziger Straße 44, 39120, Magdeburg, Germany
[i] Fraunhofer Institut für Zelltherapie und Immunologie, Perlickstraße 1, 04103, Leipzig, Germany

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**Contents**



* Corresponding author.
*E-mail addresses:* Ulrich.Sack@medizin.uni-leipzig.de (U. Sack), Andreas.Boldt@medizin.uni-leipzig.de (A. Boldt), Nora.Mallouk@chu-st-etienne.fr (N. Mallouk), Rudolf.Gruber@barmherzige-regensburg.de (R. Gruber), Krenn@patho-trier.de (V. Krenn), A.Emmanuelle.Berger@chu-st-etienne.fr (A.-E. Berger-Depincé), Karsten.Conrad@tu-dresden.de (K. Conrad), Attila.Tarnok@medizin.uni-leipzig.de (A. Tarnok), Claude.Lambert@chu-st-etienne.fr (C. Lambert), Dirk.Reinhold@med.ovgu.de (D. Reinhold), Stephan.Fricke@izi.fraunhofer.de (S. Fricke).

## 1. Introduction

Autoimmune diseases (AID) are characterized by inappropriate immune reaction against one or more of the organism's own tissues [1,2]. This is based on humoral (autoantibodies, Aab) as well as cellular (T cells) mechanisms and includes also components of the innate immunity [3]. Detection of Aab in serum is today a well-established method for autoimmunity diagnosis [4]. Besides this, cellular analyses

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in autoimmunity diagnosis are often neglected. Here, we show how deep cellular analyses today contribute to clinical diagnoses and monitoring in AID and what can be expected to enter routine labs soon. This includes the detection of autoreactive T cells, the description of immune dysregulation, the analysis of immunodeficiencies which often interplay with autoimmune phenomena, the analysis of disturbed apoptosis and DNA repair mechanisms, and investigation of genetic predisposition. Furthermore, therapy with biologicals and immunosuppressive drugs must be monitored with flow cytometry (FCM), and risks such as tuberculosis have to be excluded by IFN-γ release assays. Finally, stem cell therapy in very severe cases depends on quality assurance.

It is not possible to review autoimmune diagnostics without emphasizing the role of Aab. Detection of Aab is the key procedure in the diagnostic of AID and thus an essential part of clinical immunology. The detection of tissue bound Aab and complement components e.g. in skin or kidney biopsies using direct immunofluorescence assays (dIFA) is an important step in the diagnostic of several autoimmune disorders.

For most systemic and organ-specific AID, indirect immunofluorescence assays (iIFA) have been developed for detecting and quantifying Aab circulating in body fluids. The field of applications has increased progressively since immunofluorescence techniques were first used in 1957 to demonstrate antinuclear antibodies (ANA) [5]. It has to be noted that these methods test pathological serum on cells as substrate and are not allowing for detection of pathogenic cells of the patient.

In the past decade, several diagnostic companies have developed new technologies for automated iIFA pattern interpretation. These systems are based on digital acquisition and analysis of iIFA images by different pattern recognition algorithms and are mostly used for ANA and ANCA diagnostics [6]. Besides iIFA analysis, such systems also allow additional immunoassay analysis [7,8] but also cellular investigation such as for DNA repair (see below).

For novel autoantibodies to receptors such as aquaporin 4 or neuronal cell surface molecules, flow cytometric assays based on transfected cells have been developed (van Pelt ED, Wong YY, Ketelslegers IA, Hamann D, Hintzen RQ: Neuromyelitis optica spectrum disorders: comparison of clinical and magnetic resonance imaging characteristics of AQP4-IgG versus MOG-IgG seropositive cases in the Netherlands. Eur J Neurol. 2016;23:580-7.; van Coevorden-Hameete MH, Titulaer MJ, Schreurs MW, de Graaff E, Sillevis Smitt PA, Hoogenraad CC: Detection and Characterization of Autoantibodies to Neuronal Cell-Surface Antigens in the Central Nervous System. Front Mol Neurosci. 2016;9:37.).

## 2. Analysis of cellular infiltration of target organs

In various AID, T cells are prominent in their pathogenesis and are crucial besides or instead of Aab. Multiple sclerosis (MS), autoimmune hepatitis (AIH) and diabetes mellitus type 1 (T1D) are typical examples of strong T cell participation. In animal models and in histological samples, lymphocytic infiltration can be detected using immunostaining [1,2].

In human tissues, the diagnostic detection of lymphocytic infiltration is crucial in various autoimmune diseases. It is mostly done on a semi-quantitative scoring systems for autoimmune sialadenitis (Sjögren syndrome), rheumatic synovitis (synovitis score) [9,10], or autoimmune type A gastritis, whereas in microscopic lymphocytic colitis and coeliac disease lymphocytes are defined on a quantitative level of intraepithelial CD3 + T cells (Fig. 1) [11,12]. Furthermore, T cellular infiltration is a common finding in MS [13].

These histopathological examinations are commonly done with biopsies.

## 3. Characterization of cell populations in peripheral blood

FCM based immunophenotyping and functional analysis of peripheral blood leukocytes is one of the most important diagnostic tools in cellular laboratory diagnostics [14]. Cell functions can also be tested by culture approaches with following detection of activation, proliferation, or cytokine secretion. Furthermore, microscopy and ELISPOT assays are the most common technologies. Cells and their functions from any body fluids and effusions can be analyzed (Fig. 2). FCM allows the detection of phenotypic and functional features of individual cells at high speed and superior precision by light scatter characteristics, made more specific by applying fluorescent dye labelled antibodies or fluorescent cell function reporters like DNA dyes [15]. For antigen-specific T cell detection, multimeric technologies are promising but not yet applicable in routine diagnostics for AID [16]. ELISPOT assays allow the detection and quantification of lymphocytes secreting antibodies or cytokines by visual or semi-automated (supervised or unsupervised) image analysis [17]. Finally, batch cultures allow stimulation of cells even from whole blood and detection of various cellular and soluble readout parameters.

### 3.1. Differential diagnostics by immunophenotyping in peripheral blood

There are countless reports about cellular aberrations in AID, but only few of them have already clinical relevancy. In the supplementary material, we give an overview about findings for B, T and innate lymphocyte subpopulations, monocytes, dendritic cells, granulocytes and platelets in AID. Changes concern cell number, maturity, activation status, compartments, and homing potential (adhesion molecules and chemokine receptors). Here, we focus on established diagnostic parameters.

It is known that systemic lupus erythematosus (SLE) is occasionally accompanied by lymphopenia due to decreased T, B, and/or natural killer (NK) cells counts [18]. Cellular and functional abnormalities are also known in primary (PID) or secondary immunodeficiencies [14, 19]. FCM helps in discriminating between PID's and AID.

CVID is a common immunodeficiency and is diagnosed by decreased serum immunoglobulins. The causal relationship of low serum Ig originates from deficient memory and/or class-switched B-cells [20, 21]. Alterations in B-cell subgroups were also described in patients with SLE, but data are controversial depending on study design [22–24]. Due to the similarities in lymphocyte abnormalities between AID and PID's, an extensive clinical examination is necessary to avoid false positive/negative classification. It is known that approximately 22% of CVID patients also suffer from autoimmune symptoms [25]. Various PID patients with complement deficiencies, hyper-IgM syndromes, Adeno-lymphoproliferative syndrome (ALPS) or chronic granulomatous disease (CGD) may develop SLE-like symptoms [26]. This suggests a possible association between SLE and PID. Other autoimmune manifestations are described for patients with autoimmune polyendocrinopathy candidiasis, ectodermal dystrophy syndrome (APECED), or with immune dysfunction, polyendocrinopathy, enteropathy, X-linked (IPEX) or Good syndrome [25,27]. Associations between antibody deficiencies, variations of class-switched B-cells, and SLE are described in the current literature [20,28,29].

It should be mentioned here that another cellular markers, MHC types such as HLA-DR4 or HLA-B27, are frequently associated with Rheumatoid Arthritis (RA) or spondylitis ankylosans (SpA). They can also be detected by FCM, but this is replaced more and more by genetic methods [30].

Platelet involvement in autoimmune inflammatory processes has been extensively studied. Platelets major role is thrombosis but their involvement in atherosclerosis and vascular diseases is now well documented. Furthermore, it has been shown that platelets could have a pro-inflammatory effect under activation. In autoimmune disorders, platelets can be directly activated by Aab as shown in SSC, MS, RA, SLE, chronic glomerulonephritis and autoimmune thrombocytopenia [31]. Aab can target platelet antigens. As an example, 30% of SLE patients have antiphospholipid antibodies leading to a threefold increase in the risk for thrombotic events [32].