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INVESTIGATION OF CYTOKINE SYNTHESIS AT THE LEVEL OF INDIVIDUAL CELLS

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Abstract
The idea of localisation of antigens in tissues using antibodies was first implemented in the early 40s years. Subsequently, immunocytochemical methods became widely used in molecular clinical diagnostics, and since the mid-70s, after the discovery of monoclonal antibodies, their role has increased even more.

Keywords: immunocytochemical methods, antigens, monoclonal antibodies, molecular clinical diagnostics.

Introduction

Immunocytochemical methods allow localisation and identification of cellular components (in our case cytokines) based on their binding to antibodies. The binding site is determined using labelled antibodies or secondary labelling.

Preparations for ICCh can be of three types:

a) prints;

b) smears obtained by suitable cytological or haematological methods;

c) preparations obtained by centrifugation of cell suspensions.

The latter method is preferred when biological fluids with small numbers of cells are available to the investigator.

Tissue sections are made and incubated in a solution with antibodies to the antigen of interest (cytokine). The antibody is often bound to a fluorescent dye so that the localisation of the antigen can be seen. When the antibody finds the antigen and forms a complex with it, the free antibody is washed away and the fluorescent dye remains at the site of antigen localisation.

Using a spectrophotometer or fluorescence microscope, the localisation of the fluorescent dye can be elucidated and therefore the location of the antigen within the cell can be determined [1,3].

The reasons for the widespread use of ICCh are obvious: a wide choice of quality reagents, ease of implementation and training of personnel to record results, and in the case of light microscopy, the possibility of comparison with morphology, storage and transport of preparations, no need for highly specialised equipment.

The negative points are, undoubtedly, the subjectivity of visual assessment, the difficulty of organising quality control, the impossibility of counting a large number of cells and, as a consequence, the probability of inadequate assessment of small cell populations.

Elispot. The ELISpot method (Enzyme-Linked ImmunoSpot) is a highly sensitive modification of the IFA method that allows quantification of cells secreting a particular type of cytokine at the single cell level.

The unique high sensitivity of the ELISpot method is determined by the fact that the detectable product is on the surface of the secreting cell at the moment of analysis, being bound to its receptors. In addition, stimulation of cytokine production occurs in vitro immediately prior to detection. As a result, the ELISpot method can detect 1 cytokine-secreting cell out of 100,000 cells, which is 20 to 200 times more accurate than standard IFA.

Scheme of the ELISpot method:

1. Cytokine specific antibodies are immobilised on the bottom of a PVDF microplate.
2. Unbound sites are blocked with protein.
3. Cells with/without activator are added. During incubation, cells are activated and begin to produce and secrete cytokines that specifically bind to primary antibodies.
4. Cell washout
5. Secondary antibodies that are either enzyme conjugated or biotinylated are added.
6. If biotin is used, streptavidin conjugate is additionally added.
7. Substrate is added, resulting in a stained spot at the localisation site of the secreting cell.
8. As a result of counting the number of stains using ELISpot Reader AID, the number of cells producing a certain cytokine is determined in the samples and controls (without activator) **[1,3,6,7]**.

Cytofluorimetry. It is currently the leading method in clinical immunology. It can be used to assess intracellular cytokine production by different cell populations.

The general principles of flow cytometry involve making optical and fluorescence measurements of individual cells in a liquid light stream while they pass in a separate row through a monochromatic light, usually produced by a laser. In this case, physical properties of the cells, such as size or cytoplasmic granularity, can be measured on some individual unstained cell. Cells can also be labelled with specific dyes or a whole set of fluorochrome-conjugated antibodies directed to membrane and intracellular components of the cells **[9,10]**.

Cytofluorimetry allows to assess cytokine production at the level of a single cell using intracellular staining. The combination of intracellular and surface staining provides the opportunity to maximise the detail of information about the producing cell - class membership, subclass, functional activity, etc.. The basal level of cytokines in resting cells is rather low; therefore, cells are pre-stimulated in vitro in the presence of inducers of cytokine production and blockers of intracellular transport (brefeldin A, monensin). Surface markers are then stained, cells are fixed, permeabilised and antibodies to intracellular markers are added. The cells are then placed in the sample container of a flow cytometer and injected under pressure into the centre of a fast-moving fluid flow in the same direction through a specially designed tip, as a result of which the speed of cell movement increases dramatically and the cells line up to form a column surrounded by envelope fluid. The geometry of the tip allows to create conditions of laminar flow of the sample jet, as a result of which there is no mixing of the

suspension of the investigated cells with the flowing liquid. Getting further into the measuring chamber of the device, the cells are alternately crossed by the laser beam and excited by light of a certain wavelength. In turn, the cells send light signals of a different wavelength, which, passing through a system of optical lenses, filters, two-colour mirrors, are registered by a photomultiplier tube that converts these light signals into electrical signals processed by a computer. Two or three fluorescent signals, each reporting the reaction of a single monoclonal antibody with a specifically recognised antigen can be collected from cells together with forward (FSC-forward scatter) and side light scatter (SSC-side scatter) signals [Application 4, Figure 1]. Light scattering signals characterising cell size (FSC) and cytoplasmic and membrane features (SSC) anchor the fluorescence analysis to morphologically defined populations. The resulting data can be recorded, analysed and presented as histograms. In the case of a one-dimensional histogram, the fluorescence intensity of cells is plotted on the abscissa axis and the number of cells with a particular fluorescence intensity is plotted on the ordinate axis. By summarising the data obtained for the entire sample cell population, accurate quantitative population and subpopulation analyses can be performed **[4,5,6]**.

Thus, there are now many methods to assess the functioning of the cytokine system. Until recently, few groups of researchers were involved in cytokine studies in Russia, as biological methods are very labour-intensive and imported immunoassay kits are very expensive. With the advent of affordable domestic immunoassay kits, practitioners are increasingly interested in studying cytokine profiles.

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