MICROBIOLOGY AND IMMUNOLOGY

Immunodiagnosis of Chronic Lymphocytic Leukemia Using Immunological Chips

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The authors present their experience gained in the first clinical use of immunological biochips for detection of cellular surface antigens for immunomorphological diagnosis of chronic lymphocytic leukemia and compare the results of this method and flow cytofluorometry.

Key Words: *chronic lymphocytic leukemia; lymphocytes; CD antigens; immunological biochip; flow cytofluorometry*

Chronic lymphocytic leukemia (CLL) is diagnosed by the results of complex of studies, including morphological characterization and evaluation of the immunophenotype of pathological cells [1,4]. In addition to differential diagnostic antigens, tumor lymphocytes in CLL express other molecules on their surface membrane. Results of flow cytofluorometric studies of the expression of activation adhesion molecules in CLL were reported [5,9,10]. A specific feature of this method is simultaneous detection of just few antigens. The use of the new generation test systems, immunological biochips, simultaneously detecting tens of antigens on the cell surface will promote the solution of this problem. A biochip is a plate with immobilized antibodies specifically binding surface antigens of analyzed cells [6-8,10]. We developed biochips [10] which can be used for not only immunological analysis of cells, but also their morphological analysis, which will improve the informative value of the study.

Here we evaluated the potentialities of immunological biochips in the diagnosis of CLL.

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MATERIALS AND METHODS

We analyzed peripheral blood from 50 patients with CLL, 54% men and 46% women, mean age 68 (44-85) years. The diagnosis of CLL was made from common criteria [4]. Tumor cell immunophenotype was evaluated using original immunological biochips for detection of cell surface antigens. The immunochips were made on transparent plastic sublayer with immobilized mouse monoclonal antibodies (IgG; Sorbent Company) specific to human cell antigens: CD2, CD3, CD4, CD5, CD7, CD8, CD9, CD10, CD11a, CD11b, CD16, CD19, CD20, CD21, CD22, CD23, CD27, CD29, CD31, CD36, CD38, CD41, CD44, CD45, CD45RA, CD56, CD71, CD72, CD95, CD98, HLA-DR, IgM.

The mononuclear cell fraction was isolated by centrifugation of peripheral blood diluted (1:1) with 0.9% NaCl solution in Ficoll paque (Pharmacia) density gradient (1077 g/liter). The cells were washed thrice in 0.9% NaCl and resuspended in iso-osmotic PBS (pH 7.4) with heat-inactivated human serum (20% volume) and EDTA (0.5 mg/ml). The biochip in a container was incubated for 1 h with dry milk solution and washed in 0.05% detergent (Twin-20; Sigma Aldrich) solution, after which it was incubated (40 min) with

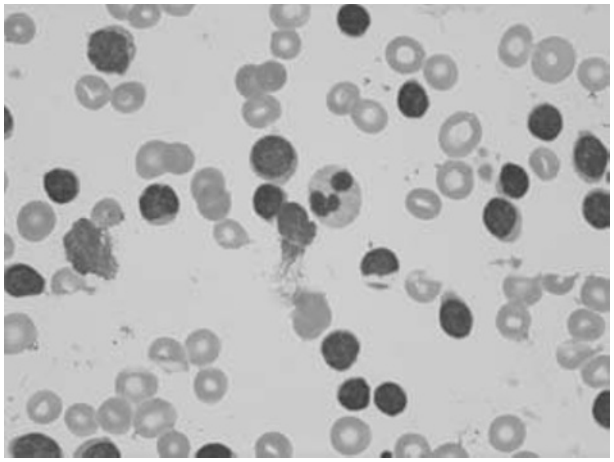

Fig. 1. Microphotograph of peripheral blood smear of patient A. (female) with CLL. Here and in Fig. 2: Romanowski–Giemza staining, ×1000.

cell suspension (5.5×10^6 cell/ml) at ambient temperature without stirring. After incubation, the biochip was washed in buffer in order to remove free cells and dried. The biochip with bound cells was then fixed in methanol (15 min) and stained. Permanent preparation was then prepared from the biochip by embedding it in Canadian balm. The biochip spots were photographed and the density of lymphocyte binding was evaluated. At least 3 fragments corresponding to 100×100 μ sites, were selected on a microphotograph of each spot. Cells in the spots were counted, which were referred to lymphoid cells by morphological signs (other cells were neglected). The results were averaged. Cell density binding in each biochip spot was expressed in percent of the maximum possible density (lymphoid cell binding density in a spot with antibodies specific to CD45 antigen was taken for 100%).

Statistical analysis was carried out using BioStat 2008 Professional statistical processing software (5.2.5.0 version). Numerical data are presented as the arithmetic mean and error of the mean ($M\pm m$). The significance of differences in quantitative signs was evaluated by nonparametric methods (Kolmogorov–Smirnov's test). Pearson's linear correlation coefficient (r) was calculated for the analysis of relationship between the signs. The results were considered significant at $p<0.05$.

RESULTS

B-cell variant of CLL was identified in all the patients. The expression of the major differential diagnostic antigens was as follows: 80.4±1.8% for CD19, 79.6±2.3% for CD20, 83.1±2.5% for CD5, 66.7±3.1% for CD23, 0.9±0.4% for CD10, 0.9±0.5% for sIgM, 16.4±2.1% for CD3, 4.6±0.7% for CD16, and 1.2±0.5% for CD56.

In 11 of 50 patients, the immunophenotype was studied only by the main differential diagnostic antigens by two methods in parallel: using immunological biochip and flow cytofluorometry (FACSCanto II flow cytofluorometer; Becton Dickinson; Table 1).

TABLE 1. Results of CLL Cell Phenotyping

Antigen	Biochip		Flow cytofluorometry	
	M, %	*m*, %	*M*, %	*m*, %
CD3	14.3	3.1	11.9*	2.0
CD4	13.0	3.7	8.1*	2.1
CD8	13.2	1.6	9.7*	1.2
CD16	2.6	0.8	2.2*	0.3
CD56	2.5	0.2	2.2*	0.4
CD19	79.6	3.1	84.5*	2.2
CD20	65.5	5.7	54.2*	6.3
CD22	50.6	8.0	46.3*	7.8
CD5	77.3	7.8	66.2*	8.0
CD23	67.8	3.1	70.8*	7.6
CD10	1.3	0.9	0.8*	0.6
CD38	36.0	8.5	30.8*	8.6
HLA-DR	82.5	4.8	76.6*	5.1

Note. *$p>0.05$.

Study of the correlation between the results of expression of the differential diagnostic antigens evaluated by the biochip and by flow cytofluorometry demonstrated a strong direct relationship (r=0.935-0.987; p<0.05).

The cells fixed in the biochip spots were similar, rather small, with lumpy compact chromatin, which characterized them as minor lymphocytes (Fig. 1).

Cells stained on the biochip had rougher chromatin structure than cells stained on the smear, which can be explained by different degree of the cell flattening (Fig. 2) [3].

High density of cell binding was noted in the spots with antibodies specific to CD5, CD19, CD20, CD22, CD23, CD29, CD44, CD45, and CD45RA and in some patients in the spots with antibodies specific to CD21, CD27, CD31, CD72, CD98, and HLA-DR. Mature lymphocytes were also detected in the spots with antibodies to T- and NK-cell antigens, but the density of cell binding was low. Normal T- and NK-cells (also present in the blood of patient A.) were bound in these spots, but significantly less intensely than the tumor B-lymphocytes. High density of cell binding in spots with antibodies to these antigens indicates that if not all the cells, at least an appreciable portion of them co-expressed these antigens. Co-expression of CD5, CD19, and CD23 antigens is characteristic of B-CLL cells [1,4].

In addition, neutrophils (were not taken into account during counting) also expressing this antigen were sometimes present in the spot with antiCD16. Cells of other types were also present, for example, myeloid cells (were not taken into account during counting). This can be explained by the fact these antigens are expressed on different cell types [2].

Hence, combined immunological and morphological study of tumor cells by immunological biochips is a modern effective method for immunological diagnosis of CLL allowing simultaneous evaluation of the expression of many antigens.

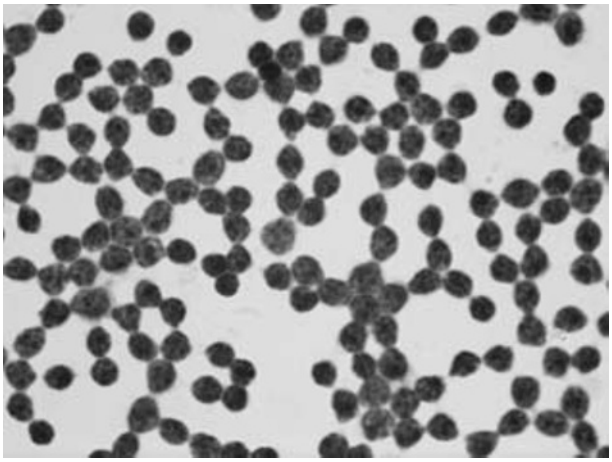

Fig. 2. Microphotograph of a fragment of biochip spot with antiCD5. By morphological signs the bound cells are mature lymphocytes.

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