Rapid Detection of Leukemia Cells by Use of a Complement-Mediated Cytolytic Reaction and an Imaging Sensor System

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We describe a system for detection of leukemia cells involving complement-mediated cytotoxic reaction and an image processing system, consisting of a charge-coupled-device image sensor, an image memory board, a personal computer, and a phase-contrast microscope. Then added to a cell suspension, monoclonal antibody specific to the fetal thymus antigen of the mouse leukemia GRSL cell produced cytosis of only GRSL cells. This cytosis decreased the brightness of the cells observed by phase-contrast microscopy. The remaining brightness was subtracted from that of the phase-contrast image of the cells before cytosis, which had been converted to a digital signal and stored in computer memory. Measurement time is 2 s. The time course for complete GRSL cytosis, as measured with this system, is 12 min; overall measurement time, including reaction time, is approximately 15 min. GRSL cells in a suspension of mixed cells were determined specifically by the system.

Additional Keyphrases: monoclonal antibodies · automated image processing · cancer detection · detection of various specific cells

Analysis of cells or tissues is developing as an attractive approach for future clinical diagnosis. Especially in cancer detection, where a diagnosis is required at an early stage, highly sensitive and rapid methods for detecting cancer cells are essential. In addition, analytes currently used for cancer detection, such as alpha-fetoprotein or carcinoembryonic antigen, are primarily applied in cases of progressive cancer.

In another study (submitted for publication), we developed a novel method for rapidly and quantitatively detecting specific cells in suspension by applying an imaging sensor system to the determination of complement-mediated lysis of cytotoxic cells with monoclonal antibody. Using this system, we could detect, within 30 min, guinea pig hepatocarcinoma L-10 cells making up 10% of a mixed-cell suspension. This 30-min cytotoxic reaction time was equivalent to that of the conventional trypan-blue exclusion test, which is based on a decrease in the selective permeability of the cell membrane, the cytotoxic reaction allowing the stain to enter the cytosolic compartment. However, because our imaging sensor system detects the phase-contrast caused by the cytotoxic reaction, we thought it possible to shorten the reaction time. Here, we report one application of the imaging sensor system combined with complement-mediated cytotoxic reaction and monoclonal antibody to the detection of mouse T-cell leukemia GRSL cells in mixed-cell suspension. We also determined the minimum essential reaction time for measurement of the cytotoxic reaction.

Materials and Methods

Cells and antibody preparation. We used murine T-cell leukemia GRSL cells (1) and guinea pig hepatoma L-10 cells (2, 3). GRSL cells were pre-incubated in RPMI1640 medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) containing, per liter, 100 mL of fetal calf serum (Mitsubishi Chem. Ind.

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Ltd., Tokyo, Japan), 2 mmol of glutamine, 10⁵ USP units of penicillin G, 50 mg of kanamycin sulfate, and 2 g of NaHCO₃. A 25-mL plastic culture flask containing the cell suspension was placed in an incubator with CO₂-enriched air (50 mL/L) and kept at 37 °C.

The L-10 cells were prepared as described in our previously submitted report. In brief, L-10 cells stored in liquid nitrogen were thawed at 38 °C, and washed three times with RPMI1640 medium. Cells were reconstituted with RPMI1640 medium (cell concentration: 6 × 10⁶ cells per milliliter).

Anti-FT-1 monoclonal antibody, which is specific for fetal thymus antigen-1 (FT-1) on GRSL cells (1), was prepared from ascite fluid by hybridoma methodology (4).

Cytotoxic reaction. The specific detection of tumor cells in this study was based on the complement-mediated cytotoxic reaction (5). Cells that carry antibodies bound to cell-surface antigens may be lysed by complement. Lysis can be followed by (e.g.) an intake of dyes such as trypan blue (6) or release of a previously introduced isotope such as ⁵¹Cr (7, 8). In this study, we monitored cell lysis by using a phase-contrast microscope. With normal cells, the difference between intracellular and extracellular compartments produces a lag-phase in the transmission of incoming light. Normal cells therefore look bright under the phase-contrast microscope, whereas damaged cells that have lost membrane integrity appear darker. In this study, applying the decrease in brightness of lysed cells, we could distinguish specific cells and cells not bound by specific monoclonal antibody. Pre-incubated GRSL cells from one flask (approximately 10⁷ cells) were centrifuged at 900 rpm (radius of gyration: 155 mm) for 8 min and decanted. The cells were washed twice with RPMI1640 medium (free of calf serum) by the same procedure and reconstituted in 1 mL of the same medium to the original cell concentration, 10⁷/mL. We then mixed 100 µL of cell suspension, 100 µL of anti-FT-1 antibody solution (10⁻⁸–10⁻¹ mg of protein per milliliter), and 50 µL of rabbit serum (Cedarlane, Ontario, Canada) in a 1.5-mL microscale centrifuge tube (Eppendorf Co., Hamburg, F.R.G.). After incubation at 37 °C, the sample was transferred into a hemacytometer (improved Neubauer type; EKDS Co., Tokyo, Japan) and analyzed with the imaging sensor system.

Instrumentation and computer hardware. Figure 1 depicts a schematic diagram of the imaging sensor system. We used a phase-contrast transmitted-light microscope (Vanox Model AHB-LB; Olympus, Tokyo, Japan) fitted with a dark-field condenser to obtain contrasting cell images. Video images of the cell suspensions in the counting chamber of the hemacytometer, obtained with a charge-coupled-device video camera (Model TI-25A; NEC, Tokyo, Japan) mounted vertically onto the microscope with a standard C-mount adapter, were displayed on a 30-cm black-and-white monitor (Model TMP-712B, NEC). After the images were focused by adjusting the focal length between the microscope objective lens and the hemacytometer, the video display was fed into an image memory board (Model FDM 98-1; Photoron, Tokyo, Japan) connected to the expanded bus of a 16-bit personal computer (Model PC-9801E, NEC). The FDM 98-1 has a 6-bit analog/digital converter, an 8-bit digital/analog converter, and 64 kilobytes of random access memory.

Computer software. The main functions of the image-digitizing program were to scan a defined frame area (256 × 256 pixels, scan time 1/60 s) of the image, express the frozen image in binary form, and record the cell image counts (number of white-level pixels) for 10 different frame scans, obtained by manually moving the field of view. Obtaining one data set (scanning, freezing, binary-translation, and completing the cell-image counts) requires about 2 s.

Measuring the time course of the cytotoxic reaction. The phase-contrast microscope was set in a thermostable chamber (Figure 1) set at 37 °C.

We mixed 100 µL of cell suspension, 100 µL of anti-FT-1 antibody solution (10⁻⁸–10⁻¹ mg of protein per milliliter), and 50 µL of rabbit serum, then promptly transferred the sample to a simplified Cunningham chamber in which a micro-slide cover glass was used as the upper slide glass of the Cunningham chamber (9). We sealed the chamber with enamel to keep the sample from drying out.

Every 1 min, a defined frame area of the image was scanned, the frozen image was converted to binary expression at a suitable threshold brightness level, and the cell-image counts were recorded.

Results

Cell number and image counts. To investigate the validity of counting cells automatically, we needed to determine the relationship between cell number and image counts. Normal (non-injured) GRSL cells, diluted to various concentrations in 0.01 mol/L phosphate-buffered isotonic saline (pH 7.4), were transferred to the hemacytometer and the contents of the cell-image area were counted by the imaging sensor system. The brightness threshold level for translating to the binary expression was fixed at 26, which was determined by the observation of the light intensity distribution map (data were not shown here). There was a linear relationship between GRSL cell number and cell image counts over the range 2.5 × 10⁶–6.5 × 10⁶ cells per milliliter (Figure 2). Thus, the number of GRSL cells could be counted automatically with the imaging sensor system.

Time course of cytotoxic reaction. To determine the minimum reaction time required for complement-mediated cytotoxic reaction in our system, we used the imaging sensor system to measure time courses of cytotoxic reactions for various antibody concentrations. In optimal conditions, the light intensity of cell image began to decrease in 4 to 5 min, reaching its minimum intensity in 10 to 15 min (Figure 3). This was only 25–50% of the time required in the conventional dye-exclusion method to indicate complete cytolyis.

The time courses for complement-mediated cell lysis of hepatocarcinoma L-10 with monoclonal antibody 3C4 were also determined (Figure 4).

Quantitative detection of GRSL cells in mixed-cell suspen-
Fig. 2. Effect of GRSL cell concentration on image counts
Threshold level, 20

discussions. On the basis of previous experiments, we measured the cell images of GRSL cells in mixed-cell suspensions 15 min after initiating cytolysis. The cell concentrations of standard suspensions containing GRSL cells and L-10 cells (FT-1 free) were adjusted to retain the same image area counts throughout. The two standard suspensions were then mixed (the GRSL cell content being the volume ratio of the two suspensions) and analyzed by the imaging sensor system. Figure 5 shows the relationship between percent GRSL cell content and image counts after treatment of the suspension with anti-FT-1 antibody and complement. GRSL cells could be detected quantitatively when they made up between 10 and 100% of the cell suspension, a linear relationship being observed between the GRSL-cells content and image area in this same range. The detectable range and linearity range were the same as that obtained for the specific determination of L-10 cells, which we have reported in the previously submitted paper.

Discussion

In our previous paper, the decrease in phase-contrast

Fig. 3. Time courses for cytoxic reaction of GRSL cells
Threshold level: 30; Antibody concentration (as protein, g/L): 1, 10^{-1}; 2, 10^{-2}; 3, 10^{-3}; 4, 0 (control)

Fig. 4. Time courses for cytoxic reaction of L-10 cells
Threshold level, 30; antibody concentration (as protein, g/L): 1, 10^{-1}; 2, 10^{-2}; 3, 10^{-3}; 4, 10^{-4}

caused by the complement-mediated cytoxic reaction was determined by the decrease in light intensity such that hepatocarcinoma cells could be specifically detected. This method can be applied to the detection of various cells if the cell-specific monoclonal antibody is available. The detection of leukemia cells in this study—and of hepatocarcinoma cells in the previous study—was specific for cell contents in the range between 10 and 100% of a mixed-cell suspension, showing that this cell-detection method is widely applicable for various target cells.

Cell lysis was very rapid, and it clearly depended on the concentration of antibody. When we used trypan-blue exclu-

Fig. 5. Specific determination of GRSL cell content (percent of total cells) in mixed-cell suspension after 15 min
Antibody concentration, 1 \times 10^{-5} 	ext{ g/L}; threshold level, 26; nonspecific cells, L-10 (guinea pig hepatocarcinoma)
A Simple Silver-Staining Technique for Detecting Bence Jones Proteins in Unconcentrated Urine

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Detection of Bence Jones proteins in urine usually involves a concentration step, followed by electrophoresis and, if necessary, immunofixation. The time-consuming and expensive concentration step can be eliminated by use of the silver-stain technique described here. This procedure, routinely used for staining unconcentrated urine, is inexpensive, sensitive, and easily performed in a clinical laboratory. Bence Jones proteins can be detected in concentrations as low as 5 mg/L.

Additional Keyphrases: electrophoresis, agarose gel • immunofixation • light chain disease • cancer • other biological fluids

Bence Jones proteins (BJP) are monoclonal light chains found in urine of patients with an immunocytoma. They occur in 70% of immunocytoma patients, 50% in conjunction with a serum paraprotein and 20% as the only paraprotein (1). BJP are also found in urine of patients with various other lymphoproliferative diseases, including Waldenström's macroglobulinemia, plasma cell leukemia, and chronic lymphatic leukemia. Detection of BJP in urine is of considerable importance in both diagnosis and prognosis of immunocytoma; it is the most important criterion for deciding whether a serum paraprotein reflects malignancy.

Most reported methods for BJP detection require specimen concentration followed by electrophoresis, with any abnormal bands being identified by immunoelectrophoresis or immunofixation. The concentration step is time consuming and expensive, especially if disposable concentrators are used. There is also a risk of contamination if concentration membranes are re-used. To overcome these problems, we have developed a silver-staining technique whereby we can analyze unconcentrated urine specimens. We have slightly modified our procedures for electrophoresis and immunofixation to take advantage of the increased sensitivity offered by the silver stain. The method we describe is simple, inexpensive, and well-suited for detection of BJP in the routine clinical laboratory.

Materials and Methods

Materials

Reagents: Agarose LSM was obtained from Litex, Glostrup, Denmark. Polyester film backing for agarose plates (GelBond) and the Sample Application Mask were obtained from FMC Corp., Marine Colloids Div., Rockland, ME; Amicon B15 Concentrators from Amicon Corp., Danvers, MA; silver nitrate from Johnson Matthey Ltd., Auckland, N.Z.; ammonium nitrate and picric acid from BDH Chemicals, Poole, U.K.; tungsbstic acid from Fluka AG, Switzerland; formaldehyde from J. T. Baker Chemical Co., Phillipsburg, NJ; and Coomassie Brilliant Blue R from Sigma Chemical Co., St. Louis, MO.