Study on Selective Single Cell Isolation Method for Blood Cells

By Tadakuni Nagasaki

Although some methods of cell isolation for culture have been established successfully, isolation of the single cell from the peripheral blood under a high magnification has not been reported so far. Some reasons are the following:

1. Difficulties in designating the living blood cell of 15 μ or more under a high magnification of phase contrast microscope.
2. Technical difficulties in selectively picking up a floating desired blood cell.
3. Difficulty in maintaining aseptic technique.
4. Difficulty in blood cell culture especially the single cell.
5. The problem of the culture chamber.

This report concerns a method for selective isolation of a single myeloblast from a patient with acute myeloblastic leukemia and its successful culture for an observation period of five days.

Materials and Methods

The peripheral heparinized blood was obtained from a patient with acute myeloblastic leukemia before treatment. The peripheral blood picture showed WBC, 50,600 with 90% myeloblasts. Bone marrow study showed presence of 77.2% myeloblasts as shown in Figure 1. Figure 2 shows a myeloblast of peripheral blood observed under the phase contrast microscope.

Procedure. Setting a Micro-Picking-Up Chamber for the Cell. Make a micropipette of about 18 μ in inner diameter and 30 mm long (see Figure 3 A) by the micro-electrode puller.* (Type PN-111) from a soft glass capillary of 600 μ in inner diameter. Dip the micropipette into the conditioned medium which will be described later and put it on the center of sterilized glass slide. Next, cover it with a sterilized coverslip (18 × 18 mm large and 0.17 mm thick) and seal three sides of the coverslip with vaselin. Setting of the micropipette before roofing by coverslip is absolutely necessary for controlling a fine pipette in such a very narrow space. Drop the leukocytes containing TC 199 at the open side of the coverslip along the micropipette from the needle (Figure 3 A). Observe under 70 × phase objective lens, using oil immersion for objective as well as condenser. In case the cell and the micropipette are not clearly seen, press the coverslip gently by using a coverslip pressor (Figure 3 B) to put the both at the same focal distance. Suck the desired cell into the micropipette very slowly employing an aspirator (one rotation gives rise to 1 mm shift to 100 ml syringe) as shown in Figure 4 A, B, and C. Then, transfer the picked cell to a modified Mackaness Chamber* containing two drops of the conditioned medium by blowing out the cell using aspirator. The chamber is made of stainless steel, 80 × 15 mm large, 1 mm in depth and has a hole of 5 mm in diameter in the center.

Observation of viability of the cell. Observe the cell in the chamber under 1,000 × magnification power with 100 × objective lens.

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Fig. 1.—One myeloblast from the bone marrow stained by the Giemsa stain.

Fig. 2.—A horse hoof-like shape of the nucleus with 2 nucleoli and scanty nuclear pattern and medium sized mitochondria crowded at one side of the nucleus are all compatible with a myeloblast (Sympon type).

Making a conditioned medium. The medium, with which Chang’s liver cells were cultivated in LH-80% and BS-20% for 2 days, was centrifuged at 3000 r.p.m. for 5 minutes. Then supernatant filtrated through 0.45μ millipore filter was mixed with original plasma and TC 199 medium in the ratio of 3:3:4 respectively as the conditioned medium to which 100 units/ml of penicillin G. and 0.1 mg/ml of dilyrostreptomycin sulfate were added.

Results

The cell within the chamber, even four days after incubation, rested on the glass floor but did not adhere to it tightly. The cytoplasm was 14μ in size, smooth in margin and narrow in width. The nucleus was generally horse-hoof shaped, but variable from time to time, and relatively scanty in nuclear pattern. The nucleolus was not clearly seen. A few medium-sized mitochondria were crowded at one side of the nucleus. These findings were compatible with a myeloblasts.1,2

During the observation, the nucleus sometimes rotated clockwise or counterclockwise and also the cytoplasm moved slightly as a whole. Figure 5 shows sequence of pictures taken at every about 30 seconds for 450 seconds indicating a clockwise rotation of nucleus from 11 o’clock to 6 o’clock.

On the fifth observation day, the cell started to show degenerative signs such as condensation of the nuclear membrane and nucleus, swelling of the mitochondria, and no longer movement of the nucleus and the cytoplasm.
Fig. 3.—Schematic drawing of picking-up chamber for the cells. (A) Drop cells containing medium at the open side of the coverslip along micropipette through the needle; (B) Side view of the same with coverslip pressor.

Fig. 4.—Sucking myeloblast into micropipette: (A) A myeloblast just entering into the mouth of micropipette; (B) The same, having entered just through micropipette; (C) The same, situated at the proper position for transferring.

Finally, the cell died and floated itself in the medium. In this study, mitosis was not observed and there was found no change in pH of the medium.

**Discussion**

Picking-up methods for fresh living blood cells have been invented by several researchers. Hanging-drop method was employed by Furth et al.8
Fig. 5.—Sequence of changes in a myeloblast cultivated for 120 hours after picking up. Each picture was taken about every 30 seconds. 1,000× (100× objective), Kodak* Tri-X Pan film. Looking through this sequence, note a rotation of about 180° of the nucleus by the direction of indentation.
in 1937 for the mouse leukemic cells, then by Nossal in 1958 for the lymph node cells of the rat and by Attardi in 1959 for the lymph node cells of the rabbit. This hanging-drop method, however, have shown several disadvantages for my study: (1) difficulties in precise designation of the cell because of insufficient magnification without use of oil condenser, (2) difficulties in picking up a given floating cell, (3) possibility of contaminating of many undesired cells into the micropipette, and (4) requirement of much time and energy to detect a desired cell in the medium extremely diluted in cell number. De Fonbrune (1949) picked up cultivated or incubated cells and microorganisms in his oil chamber by micro-manipulator. His picking-up method falls under the category of hanging-drop method because the material containing a droplet fluid is located undersurface of the coverslip, thus indicating unsuitable for my study.

To cover these disadvantages, many attempts were tried to obtain in this work and finally, a very thin picking-up chamber, so-called micro-picking-up chamber, has been invented. To this chamber a 70 X phase objective lens with oil immersion condenser is applicable for making the cell more discernible, and a given cell is easily suck into the micropipette from the cells stretched out in a monolayer state in the chamber. In addition, it was found that a large syringe of 100 ml aspirator is favorable to use in this experiment instead of a small (2 ml) syringe originally attached to the Leiz micromanipulator.

According to the literature, the blood cell culture has been the most difficult problem. Many workers have engaged in blood cell culture on normal and leukemic blood, but the long-term cultures became fibroblastic with no recognizable leukemic cells remaining. Iwakata et al. in 1964, however, succeeded in long-term culture of leukemic cells of a patient with acute myelogenous leukemia. But this was done by mass culture. They emphasized in their study the need of “irradiated feeder layer” for initiating culture medium.

In 1948, Sanford et al. isolated single cells from cultures of a strain L in Carrel flask by use of a micropipette and succeeded in growing the single cells with a conditioned cell-free medium. They emphasized that single cell can adjust with a culture medium already conditioned by the growth of large culture of living cells. Burrow and Moen also stated that single cells can grow only in the presence of other tissue cells where metabolic products act as a stimulus for proliferation.

Puck used x-irradiated “feeder” cells to supply conditioning factors for growth of clones from single cells. The “feeder” layer was not applied in my experiment because the chamber was so small.

In this experiment, structural modification due to the culture such as fibroblastic metamorphosis was not observed at least under the phase contrast microscope. The viability of the isolated cell for five days has been evidenced by attaching state of the cell on the glass floor, the clear-cut nucleus and nuclear rotation during observation. The average time for the nuclear rotation through 180 was about 450 seconds. Pomerat described the nuclear
rotation in tissue cultures of epithelium from the adult human nasal mucosa and cells of the HeLa strain by Leone et al. Similarly, Hintzsche found nuclear rotation in cultures of epithelium from the young mouse kidney and Nakai also, in ganglion cells from the dorsal root of culture embryos. The long-term culture of single cell picked up in this manner, however, remains as another unsolved problem which may need investigation on culture medium, cell number, cell source, feeder layer, etc.

**SUMMARY**

In this study a method of selective isolation of a human leukemic cell was described. The method consisted of (1) use of a micro-picking-up chamber with a very small micropipette (18μ in inner diameter), and coverslip pressor, and (2) use of a 70× phase objective lens with oil immersion condenser.

As an evidence of viability of the isolated cell, the nuclear rotation was demonstrated in a conditioned medium for five days culture.

**SUMMARIO IN INTERLINGUA**

Es describite un metodo pro le isolation selective de human cellulas leucemic. Le metodo consiste de (1) le uso de un camera microinceptori con un micrissime micropipetta (de un diametro interior de 18 μ) e un coperi-objecto como pressor e (2) le uso de un lente 70× de objectivo phasic con condensator a immersion in oleo.

Como evidentia del viabilitate del isolate cellulas, le rotation nucleari esseva demonstrate in un medio conditionate pro un cultura de cinque dies.

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