วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI
ASSOCIATED MEDICAL SCIENCES

Vol. 7 No. 3
September 1974

RAPID SEPARATION OF LYMPHOCYTES BY COTTON FIBER ADHERENCE TECHNIQUE

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ABSTRACT.

The new modification of the surface adherence technique for separation of lymphocytes from peripheral blood including the utilization of cotton fiber in place of nylon wool and the elimination of plasma trapping volume by using the plastic syringe instead of test tube was tested in 26 blood samples. The highly purified lymphocyte population ($83.30 \pm 14.79\%$) with $60-70\%$ of the original lymphocytes and normal ratio of lymphocyte subpopulations was obtained comparable to those separated by the density gradient

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centrifugation (Ficoll-Hypaque) technique. The additional advantages of this technique are the shortest time required, the elimination of excessive manipulation of cells and chances of bacterial contamination.

INTRODUCTION.

At present there are a number of methods by which lymphocytes essentially free of granulocytes and red blood cells contamination can be obtained from venous blood for use in various immunological studies. Three distinct steps are usually involved: sedimentation of red blood cells; separation of granulocytes from lymphocytes; and removal of remaining red blood cells. The most important step is the removal of granulocytes from lymphocytes which can be achieved by: surface adherence technique using the nylon fiber (1, 2), cotton (3,4), glass wool (5,6), glass (7,8) or polysterene beads (9). wide-surfaced containers (10, 11); phagocytosis of carbonyl iron filings followed by centrifugation or by introducing a magnet into the cell suspension (12, 13); density gradient technique using albumin (14, 15), dextran, methyl cellulose, Ficoll (16) or Hypaque (17). These methods are not always entirely satisfactory because they are not only time-consuming but also require large quantity of blood sample. These obstacles are partly overcomed by the simplified density gradient centrifugation technique using Ficoll-Hypaque mixture (18).

We are describing the usefulness of the modified surface adherence technique using cotton fiber which is very simple and the highly purified lymphocyte suspension can be obtained within 15 minutes.

MATERIAL AND METHODS.

Leukocyte-rich plasma: Ten milliliters of venous blood samples

from 26 adult volunteers were collected into the heparinized sterile plastic syringe. The leukocyte-rich plasma samples were prepared by the dextran sedimentation technique (19).

Lymphocyte separation: Cotton-fiber adherence technique: Leukocyte-rich plasma sample was transferred into a sterile plastic syringe (5-10 ml. capacity) containing 200-300 mg. of sterile cotton fiber. After 15 minutes of occasional inversion the plasma sample was expressed from the syringe. The total plasma volume, leukocyte count, differential count and trypan blue viability test (20) were carried out before and after the procedure.

Density gradient centrifugation technique: Two milliliters of leukocyte-rich plasma sample was gently placed over the 3 ml. of a freshly-prepared mixture of 24 parts of 9% Ficoll (Pharmacia, Uppsala, Sweden) and 10 parts of 34% Sodium diatrizoate (Hypaque, Wintrop Laboratories, N.Y.). After centrifugation at 150 x g for 15 minutes, the lymphocyte-rich layer at the interphase was collected, washed 3 times with Seligmann's balanced salt solution (16) then resuspended in the AB serum.

The percentages of B and T lymphocytes in plasma samples were determined by the rosette formation technique (21).

RESULTS.

1. The average percentage of lymphocytes in 26 plasma samples tested was $39.34 \pm 8.92\%$, and those obtained from cotton fiber adherence technique and Ficoll-Hypaque density gradient technique were $83.30 \pm 14.8\%$ and $95.92 \pm 2.77\%$ respectively.

2. There is no significant difference between the percentages

of B and T. lymphocytes in the blood samples obtained from these 2 methods.

3. The percent recovery of lymphocytes of both methods are comparable, between 60 - 70% of total lymphocytes in the original sample.

COMMENTS.

Highly purified lymphocyte population in the test system is essential for most the immunological studies. Various methods for purification of lymphocyte suspensions have been described. Of these, the two most widely used are the glass or nylon wool adherence technique (1, 2, 5, 6) and the density gradient centrifugation using Ficoll - Hypaque mixture (17, 18, 22). Although the former method is more feasible than the latter, the serious disadvantage of B - lymphocyte loss secondary to the adhesion of these cells to the nylon fiber renders it suitable only for certain type of studies. Our modification of the cotton fiber in the syringe not only eliminate the B lymphocytes loss but also minimize the total lymphocytes loss secondary to large trapping volume within the cotton or nylon fiber mass as observed in the conventional method (4). In addition, this particular method required only 15 minutes (compared to 2 - 3 hours when the Ficoll - Hypaque technique is used) with minimal manipulation of cells and chances of bacterial contamination and also much less expensive.

CONCLUSION

Highly purified lymphocyte population are successfully prepared from peripheral blood by the cotton fiber adherence technique. The main advantages of this technique are the shortest time required, minimal cell manipulation and

chance of bacterial contamination, higher rate of lymphocyte recovered while the ratio of its subpopulations remain unaltered.

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ย่อเรื่อง

คณะผู้ทดลองได้ศึกษาและปรับปรุงวิธีใหม่ในการแยก Lymphocytes จาก peripheral blood แบบ surface adherence ด้วยการใช้ cotton fiber แทน nylon wool ซึ่งดูดซับเอา plasma ไว้มากและใช้ plastic syringe แทนหลอดแก้วธรรมดา

จากผลการตรวจเลือดตัวอย่าง 26 ราย พบว่า purified lymphocyte population ที่แยกได้มีค่าเฉลี่ยสูงถึง 83.30 ± 14.79 % จากปริมาณ 60-70 % ของ original lymphocytes

ข้อดีของวิธีแยก Lymphocytes ด้วย cotton fiber นี้ก็คือกินเวลาน้อยลดอัตราทั้งการสูญเสีย cells และอัตราของ bacterial contamination นอกจากนี้ยังพบว่า normal ratio ของ Lymphocyte subpopulation ที่แยกได้เมื่อนำไปเปรียบเทียบกับ lymphocytes ที่แยกโดยวิธี density gradient centrifugation (Ficoll-Hypaque) ปรากฏว่าผลที่ได้ไม่แตกต่างกันเลย