A Closed System for Large-Scale Lymphocyte Purification

By A. H. Woods

The isolation of pure suspensions of human lymphocytes is required for the production of antilymphocyte globulin,\textsuperscript{1,2} for typing of histocompatibility factors found to reside on the lymphocyte,\textsuperscript{3} and for the study of immunological reactions found to be reproducible in vitro.\textsuperscript{4} With this impetus, a number of methods have been devised for separating lymphocytes from other cells in peripheral blood. Of these, the most successful have been those based upon the failure of lymphocytes to attach to glass or other inert surfaces, while most other cell types adhere to some degree. The use of columns packed with glass wool was introduced by Johnson and Garvin,\textsuperscript{5} and advanced by Rabinowitz\textsuperscript{6} who substituted siliconized glass beads. Walford\textsuperscript{7,8} used similar columns packed with nylon cotton. These methods are successful in securing lymphocyte preparations of high purity. However, the columns are cumbersome when used for large scale preparation. In addition, there is considerable cell loss due to the trapping of lymphocytes on the columns. To avoid these problems the system described in this report has been developed.

Materials and Methods

Plastic blood bags of 600-ml. and 300-ml. capacity, together with a vacuum-operated aspirating and mixing device designed for them (Hemolator), were obtained from Fenwal. Nylon powder (Polypenco Type 66-D) was obtained from DuPont, courtesy of Dr. J. Zimmerman. Plasmagel, a 3 per cent gelatin solution, was obtained from Lab. Roger Bellon, 159 Ave. du Roule, Neuilly, France.

The procedure consists of two steps: first, the sedimentation of lightly heparinized blood mixed 2:1 with Plasmagel, and second, the transfer of the plasma supernatant into a second plastic bag containing nylon powder and a few glass beads where it is agitated until a clot forms. This clot effectively removes platelets, granulocytes, and monocytes in a nylon-fibrin matrix which forms into a tight ball as clot retraction proceeds. The resulting lymphocyte suspension may now be decanted. The maximum quantity of blood and Plasmagel that may be placed in a 600-ml. bag and still permit adequate sedimentation is about 300 ml. For the 2:1 blood–Plasmagel ratio adopted, this would include 200 ml. of blood and 100 ml. of Plasmagel containing 100 units of heparin. The amount of heparin is adjusted to 0.5 units per milliliter of blood to obtain a degree of anticoagulation which will permit a 40 min. sedimentation period without clotting, and yet clot vigorously during the second step of agitation with nylon and glass beads. This proportion of heparin to blood is quite critical. The calculated amounts of Plasmagel and heparin are then inserted into the bag, about 15 ml. being reserved for rinsing the collecting tube, and the bag is placed in the Hemolator. A gentle mixing action is begun, a vacuum of 20 in. is applied to the bag, and a routine venepuncture performed. Aspiration of blood is monitored.
by weight. After collection, the tubing is rinsed with the reserved portion of Plasmagel and clamped. Sedimentation is achieved by hanging the bag in a plasma extracting unit (Fenwal) at 37°C for 40 min. A small amount of air is left above the blood. After sedimentation, the plasma supernatant is expressed slowly into a 300-ml transfer bag which has been prepared in advance with 200 mg. of nylon powder per 100 ml. of blood (the exact amount need not be precise) and a few glass beads to seed the clot. This bag is now inserted into the Hemolator and allowed to distend with air. A vigorous mixing action is begun and continued for 30 min. During this time a clot may be seen to form slowly and knit into a ball about the size of a marble. At the end of 30 min., the lymphocyte suspension may be decanted through the collecting tube for further use. A characteristic nylon–fibrin ball is shown in Fig. 1.

RESULTS

This method has been tested on blood samples from 18 donors; a total of 45 separate purifications. The results are listed in Table 1. Average degree of purity achieved was 96 per cent, the chief contaminants being polymorphonuclear granulocytes (two per cent) and eosinophils (two per cent). There are approximately two erythrocytes for each lymphocyte in the final preparation.
Table 1.—Yield of Lymphocytes from 100-ml. Blood Samples by Nylon Powder Method (11 experiments)

<table>
<thead>
<tr>
<th></th>
<th>Whole Blood</th>
<th>Lymphocytes ( \times 10^6 )</th>
<th>After Sedimentation</th>
<th>After Nylon</th>
<th>Final Yield (%)</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td>256.7</td>
<td>163.2</td>
<td>151.3</td>
<td>59.3</td>
<td>95.82</td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td>54.0</td>
<td>32.8</td>
<td>32.7</td>
<td>4.2</td>
<td>2.62</td>
<td></td>
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<tr>
<td>Standard Deviation</td>
<td>(161-352)</td>
<td>(116-228)</td>
<td>(104-209)</td>
<td>(46-65)</td>
<td>(89-98)</td>
<td></td>
</tr>
</tbody>
</table>

Recovery of lymphocytes from the original blood was over 60 per cent. Two subjects, not included in Table 1, gave repetitively poor results. One had received x-ray therapy and the second was on prednisone. Their defect in granulocyte adherence would appear to be only partial, since lymphocyte purification of 95 per cent was achieved in both subjects by the nylon cotton method. Viability of the separated lymphocytes was established at 100 per cent by trypan blue exclusion. Morphological integrity was shown by phase contrast, Wright’s stained smears, and acridine orange fluorescence. In addition, viability was established by the survival of cultured cells, 82 per cent surviving after one week in TC 199 reinforced with 15 per cent fetal calf serum. When phytohemagglutinin (PHA-P) was added to the cultures, a typical blastogenic reaction involving about 95 per cent of the cells occurred after 72 hr.

The influence of particle size of the nylon powder was investigated. Of eight mesh sizes available for trial, only one was successful. The critical size appears to be about 70 mesh; larger particles do not appear to provide the proper surface for reasons which are not clear.

The removal of nylon particles from the final lymphocyte suspension was essential for the work in this laboratory, where the increase in cell volume with blastogenesis is used as one of the criteria of in vitro lymphocyte reactivity. Screening for nylon was done by acridine orange staining, in which the nylon fluoresces a brilliant green. It was found that the fibrin network which develops in the transfer bag containing nylon is 100 per cent efficient in removing nylon particles.

**Discussion**

The nylon powder method for lymphocyte purification described here appears to have several advantages over established methods. It is, first of all, extremely simple and technical personnel can be trained in its use with ease. There are few reagents to prepare, and these can be conveniently made in advance and stored indefinitely. The system is portable and may be taken wherever specimens are to be collected. Its chief advantages, however, lie in the yield of lymphocytes and the large volumes of blood which may be processed for work in which large numbers of lymphocytes are required. It is a simple matter to operate two or more Hemolator units from the same donor, allowing over 500 ml. of blood to be processed in one operation. The yield consistently runs over twice that of the column methods as illustrated in Table 2, where the lymphocyte losses occurring in the different methods.
Table 2.—Comparison of Lymphocyte Purification Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Experiments</th>
<th>Loss of Lymphocytes During:</th>
<th>Final Yield (%)</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sedimentation Step (%)</td>
<td>Adherence Step (%)</td>
<td></td>
</tr>
<tr>
<td>Glass Beads (6)</td>
<td>18</td>
<td>50.7</td>
<td>16.9</td>
<td>32.4</td>
</tr>
<tr>
<td>Nylon Cotton (8)</td>
<td>6</td>
<td>49.7</td>
<td>35.0</td>
<td>15.3</td>
</tr>
<tr>
<td>Nylon Powder</td>
<td>11</td>
<td>36.4</td>
<td>7.3</td>
<td>59.3</td>
</tr>
</tbody>
</table>

are compared. The gain in recovery with the nylon powder method may be partly attributed to more efficient sedimentation in plastic bags. However, the major gain is in the adherence step. Columns inevitably trap lymphocytes which can only be recovered by the use of large column washes at the risk of contamination. This problem is avoided by the formation of the fibrin–nylon matrix which serves the triple purpose of removing granulocytes, removing nylon, and expressing unadherent lymphocytes by the process of clot retraction.

**SUMMARY**

A method for lymphocyte purification is described. This is a closed system in which lightly heparinized blood is sedimented with Plasmagel in a plastic blood bag. The plasma layer is transferred to a second bag containing nylon powder and clotting is induced by agitation. Platelets, polymorphonuclear granulocytes, and monocytes are removed and trapped in the clot, together with the nylon powder. Lymphocyte suspension of 96 per cent purity and 61 per cent overall yield are produced. Viability of the lymphocytes is excellent and they are shown to be suitable for tissue culture. Comparison with other methods for lymphocyte isolation show this method to give higher yields and to be simpler in performance. It is particularly adaptable for large volumes of blood.

**ACKNOWLEDGMENT**

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**REFERENCES**

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LARGE-SCALE LYMPHOCYTE PURIFICATION


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