# **UNIT 7.24** | **Isolation of Human Basophils**

Human basophils are normally the rarest of all circulating leukocytes (there are  $\sim 2\text{-}8 \times 10^4$  basophils/ml blood) and thus are among the most difficult of the blood elements to purify. In the following protocol, basophils are isolated from leukocyte populations, resulting in preparations of cells of which 5% to 50% are basophils and the rest are lymphocytes along with neutrophils and monocytes. This technique is advantageous because of its speed and because it minimizes basophil stimulation and histamine release.

*CAUTION:* When working with human blood, cells or infectious agents, biosafety practices should be followed (see Chapter 7 introduction).

# BASIC PROTOCOL

#### BASOPHIL ENRICHMENT BY PERCOLL GRADIENT CENTRIFUGATION

#### Materials

Venous blood, freshly drawn

0.1 M EDTA, pH 7.7

Percoll gradients in 15-ml polystyrene conical tube (support protocol)

Hanks balanced salt solution (HBSS; without Ca<sup>++</sup>, Mg<sup>++</sup>, and phenol red; *APPENDIX* 2)

50-ml polypropylene conical tubes

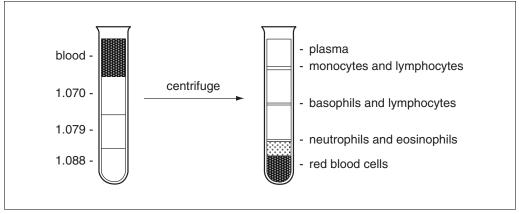
Beckman TJ-6 centrifuge with TH-4 rotor (or equivalent)

1-ml disposable polypropylene pipets

15-ml polystyrene conical tubes

Additional reagents and equipment for blood collection and Wright-Giemsa cell staining (APPENDIX 3)

- 1. Place 40 ml unheparinized venous blood (immediately after it is drawn) into a 50-ml polypropylene conical tube containing  $\frac{1}{10}$  the blood volume of 0.1 M EDTA, pH 7.7.
  - Alternatively, the blood may be diluted in a similar manner with HBSS containing 10 U heparin/ml (preservative-free).
- 2. Layer 4 ml blood mixture over each of 10 Percoll gradients and centrifuge 25 min at  $1200 \text{ rpm} (300 \times g)$ , room temperature, with no brake.
- 3. Gently remove the gradients from the centrifuge. Remove each cell band (Fig. 7.24.1) with a 1-ml polypropylene disposable pipet and pool similar bands from each



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**Figure 7.24.1** Percoll gradient before and after centrifugation. Percoll solution densities are expressed in g/ml near the gradient before centrifugation.

gradient into a clean 15-ml polystyrene conical tube. Once each band is pooled, remove 3.3 ml aliquots and place in separate 15-ml conical tubes.

Although the majority of basophils are usually found in the middle band, basophil density varies from donor to donor and the basophils may be present in other than the expected band.

4. Wash cells of each aliquot twice as follows: add 10 ml HBSS, mix, centrifuge 7 min at 1200 rpm  $(300 \times g)$ , 4°C, and discard supernatant.

The basophil population can be further purified as described in critical parameters.

5. Stain an aliquot from each preparation with Wright-Giemsa stain and examine microscopically to establish identity of basophils (see anticipated results, *APPENDIX 3*).

Most basophils stain dark reddish purple.

## PERCOLL GRADIENT PREPARATION

## **Additional Materials**

Percoll (Pharmacia)

10× HBSS without Ca<sup>++</sup>, Mg<sup>++</sup>, and phenol red (APPENDIX 2; prepare ingredients at 10-fold higher concentration)

0.25 M HEPES, pH 7.3

1 M HCl

15-ml polystyrene conical tubes

1. Prepare a stock Percoll solution in a 250-ml glass beaker by mixing the following:

90 ml Percoll 9 ml 10× HBSS 1 ml 0.25 M HEPES buffer, pH 7.3 0.4 ml 1 M HCl.

The pH of the resulting solution should be 7.4.

|                | Percoll solution/ |
|----------------|-------------------|
| Density (g/ml) | 1× HBSS (ml/ml)   |
| 1.070          | 24.0:20.0         |
| 1.079          | 27.0:15.9         |
| 1.088          | 23.0:10.0         |

2. Carefully prepare Percoll density solutions for 10 gradients as follows:

Prepare solutions the day of the experiment and discard any unused portions. Verify density of the solutions by measuring refractive index (RI) at 22°C with a refractometer. Plot density versus RI and determine the slope of the straight line. A change in density of  $10^{-3}$  g/ml corresponds to an increment of  $1.5 \times 10^{-4}$  in RI.

3. Form the final discontinuous Percoll gradient in each of ten 15-ml polystyrene conical tubes by sequentially adding to the bottom of the tube 4 ml of 1.070 g/ml Percoll density solution, 4 ml of 1.079 g/ml solution, and 3 ml of 1.088 g/ml solution.

Solutions may be layered sequentially using a 5-ml syringe with a 19-G spinal needle, or with a column pump equipped with 3-mm tubing (narrowed at the end by insertion of a piece of thin polyethylene tubing). As the more dense solutions are added to the bottom of the tube, the less dense solutions rise up the tube, forming the final gradient.

PROTOCOL

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Immunologic Studies in Humans

#### COMMENTARY

# **Background Information**

Basophils comprise <2% of erythrocytefree leukocyte suspensions. Nevertheless, they can be studied in these suspensions by taking advantage of the fact that basophils are the only cells in the peripheral blood that contain histamine and for which cell function can be monitored by measuring histamine release (Dembo et al., 1979). However, it is sometimes desirable to use preparations that have been enriched for basophils, particularly for mediator studies. This enrichment can be accomplished by Percoll gradient centrifugation, which takes advantage of the uniform density of basophils. The technique presented here (taken from Leonard et al., 1984, as adapted from Raghuprasud, 1982) has proven to be both simple and reproducible.

Basophil function can be assessed by measuring the release of histamine following exposure of the basophil-rich cell suspensions to degranulating stimuli. Such stimuli include antibodies to the IgE molecule or specific antigen recognized by specific IgE on the basophil surface (Dembo et al., 1979).

# Critical Parameters and Troubleshooting

The percentage of basophils obtained can vary considerably between donors. A consistently higher degree of basophil purity can be achieved by selecting donors who give higher percentages of basophils in the middle band (Fig. 7.24.1).

Percoll gradients must be carefully constructed to avoid mixing the layers. After centrifugation, the layers must be removed with care to minimize mixing of cell populations. The basophil layer should be removed as soon as centrifugation is finished. In addition, the speed and temperature of centrifugation are critical and should be set as indicated.

Contaminating small lymphocytes can be removed from Percoll gradient–purified cells by passing cells through a glass bead column (Rabinowitz, 1964). Basophils attach to the beads, but lymphocytes pass through. The basophils can then be recovered by adding EDTA to the column (Pruzansky and Patterson, 1981). Percoll gradients can also be combined with benzylpenicilloyl (BPO) affinity techniques (MacGlashan and Lichtenstein, 1980; Warner et al., 1987) if further purity is required.

As can be seen in Figure 7.24.1, basophils in the basophil-lymphocyte band have a density

between 1.070 and 1.079 g/ml. A shift of ~1% of the cell density would determine if the cells localize in the monocyte-lymphocyte layer or in the basophil-lymphocyte layer. Thus, small differences in the composition of the medium affect results.

Venous blood mixed with EDTA leads to better recovery than if blood is mixed with heparin. Blood should not be held at room temperature longer than required as it may result in more basophils localizing in the monocyte-lymphocyte layer.

# **Anticipated Results**

The monocyte-lymphocyte layer is dense and highly cellular. It is separated from the basophil-lymphocyte layer by a clear or slightly opalescent zone. The basophil-lymphocyte layer is faint but well-defined and is separated from the neutrophil-eosinophil layer by a clear-to-opalescent layer. This last layer may contain detectable RBC (depending on the donor), as may the zone between the neutrophil-eosinophil layer and RBC pellet.

Through selection of donors and repetition of this protocol, populations consisting of 10% to 20% basophils may be reproducibly obtained from the basophil-lymphocyte layer.

#### **Time Considerations**

The entire procedure takes 3 to 4 hr to complete, including preparing gradients and centrifuging and washing basophils. Initial setup time, however, requires an additional 1 to 2 hr to prepare stock solutions. Gradient preparation takes ~30 min and should not be hurried.

#### Literature Cited

Dembo, M., Goldstein, B., Sobotka, A.K., and Lichtenstein, L.M. 1979. Degranulation of human basophils: Quantitative analysis of histamine release and desensitization of a bivalent penicilloyl hapten. *J. Immunol.* 123:1864-1868.

Leonard, E.J., Roberts, R.L., and Skeel, A. 1984. Purification of human blood basophils by single step isopycnic banding on Percoll. *J. Leukocyte Biol.* 35:169-177.

MacGlashan, D.W. and Lichtenstein, L.M. 1980. The purification of human basophils. *J. Immunol.* 124:2519-1521.

Pruzansky, J.J. and Patterson, R. 1981. Enrichment of human basophils. *J. Immunol. Methods* 44:183-191.

Isolation of Human Basophils Rabinowitz, Y. 1964. Separation of lymphocytes, polymorphonuclear leukocytes and monocytes on glass columns, including tissue culture observations. *Blood* 23:811-814.

Raghuprasud, P.K. 1982. A rapid simple method of basophil purification by density centrifugation on Percoll. *J. Immunol.* 129:2128-2133.

Warner, J.A., Reshef, A., and MacGlashan, D.W. 1987. A rapid Percoll technique for the purification of human basophils. *J. Immunol. Methods* 105:107-110.

## **Key Reference**

Leonard et al., 1984. See above.

This reference is the principle source of this technique and describes results obtained from repetitive applications of the procedure.

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