METHOD OF ISOLATING SMALL LYMPHOCYTES FROM THE PERIPHERAL BLOOD

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A method of obtaining small lymphocytes from human peripheral blood, yielding a 95-98% suspension of these cells, is described. Immunization of rabbits by the suspension of lymphocytes prepared in this way can be used to obtain an active lymphocytotoxic serum.

When immunizing animals in order to obtain highly specific immunocytotoxic sera it is desirable to use small lymphocytes from the patient who is to receive the graft. In such cases it is most convenient to obtain these lymphocytes from the peripheral blood.

However, because of the small number of small lymphocytes in peripheral blood great difficulty is experienced in isolating them.

In the investigation described below, two methods were used in succession for this purpose: that suggested by Coulson and Chalmers for isolating lymphocytes from peripheral blood and Holub's method [3] as modified by Kraskina and co-workers [1] for isolating small lymphocytes from splenic tissue.

In silicone-treated sterile flasks, 200-300 ml defibrinated blood (it is better to use a defibrinator than beads, so as to avoid frothing) is mixed with 3% gelatin solution in physiological saline in the ratio of 3:1, i.e., 3 parts blood to 1 part freshly prepared gelatin solution. It is recommended [3] that the gelatin be dissolved in warm (not hot) physiological saline; the temperature of the gelatin solution during mixing with the blood must not exceed 37°. The prepared gelatin solution is sterilized by passing it through a Seitz filter. The mixture of blood and gelatin is incubated for 30 min at 37°.

During incubation the contents of the flasks separate into a top layer, containing all the lymphocytes, eosinophils, and a few neutrophils and erythrocytes, while the bottom layer contains erythrocytes and a large proportion of the granulocytes. With a sterile syringe the top layer is removed and put aside for further treatment, while the bottom layer can be injected into the patient, observing complete sterility (tested on 3 donors).

In order to pass on to the next stage of the work, two sucrose density gradients must be prepared. The first consists of successively layered 40, 30 and 20% solutions, and the second of 25, 20, and 15% sucrose solutions made up in Hanks's solution. The sucrose solutions were layered into 250-ml flasks. The height of each layer is 15-20 mm. The top layer, containing the majority of the lymphocytes, is carefully applied on to the surface of the first gradient (the height of this layer above the sucrose may be up to 30-40 mm) and centrifuged for 8-10 min at 700-800 rpm (type TsLR-1 centrifuge).

After centrifugation the contents of the flask separate into 5 distinct layers. Each layer is removed separately with great care by means of a syringe or Pasteur pipet, and studied in the native form under the microscope to determine its cell composition. Usually the 2nd, 3rd, and 4th layers are riches in small lymphocytes, the content of which may reach 80-90%, the other 10-20% consisting of large lymphocytes, monocytes and granulocytes. All layers contain traces of erythrocytes. To obtain a purer suspension, the

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small lymphocytes from the 2nd, 3rd, and 4th layers are washed once or twice with Hanks's solution and layered on the second sucrose density gradient. This is centrifuged under the same conditions for 7-8 min, after which 5 layers are again formed in the flask; the 2nd, 3rd, and 4th layers contain 95-98% of small lymphocytes and small numbers of erythrocytes as contaminants. The layers with small lymphocytes are mixed in one flask, diluted with a large volume of Hanks's solution, and centrifuged at 1500 rpm for 20 min, for these cells do not sediment readily. After removal of the greater part of the supernatant the residue is shaken to give a uniform suspension, and the number of cells is counted in a Goryaev's chamber.

Hemolysis of the erthrocytes was produced by a 1% solution of hydrochloric acid (sp. gr. 1.19), a few drops being added (until decolorization, if the contamination by erythrocytes was negligible, or until slight darkening if they were more numerous). In this way, 5000 small lymphocytes in a volume of 1 mm^3 were obtained from 200 ml blood, provided that they were concentrated in 5 ml Hanks's solution.

The isolated small lymphocytes were used as antigen for immunization of rabbits. Four rabbits weighing 2.8-4 kg were taken, and 1 ml of the lymphocyte suspension in Hanks's solution or physiological saline (1 ml solution contained an average of 5×10^6 small lymphocytes) was injected into the auricular vein. An injection of the suspension of small lymphocytes was given every 5th day for 5 injections. On the 7th day after the last injection blood was taken from the auricular vein for determination of the antibody titer. This titer was determined by the complement fixation reaction, using small lymphocytes as antigen. All the immunized rabbits produced antibodies against small lymphocytes in titers of 1:80 (+++ and ++), 1:160 (++), and 1:640 (+++).

By means of the combination of methods described above it is thus possible to isolate from peripheral blood an almost pure fraction of small lymphocytes with minimum blood loss.

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