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Storage of lymphocytes for immunological study

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It not uncommonly happens that tests of lymphocyte responsiveness to antigens cannot be carried out immediately so that a simple means of storage is useful. This is especially the case with the electrophoresis method described by Field and Caspary (1970) where unpredictable vagaries of the instrument add to the pressure of numbers. Simple procedures are described to allow testing to be delayed.

Method

Lymphocytes were separated from fresh venous blood by the modification introduced by Hughes and Caspary (1970) of the method devised by Coulson and Chalmers (1967). The method utilizes carbonyl iron and methyl cellulose and gives yields of approximately 10^6 lymphocytes per ml blood, 95-98% small and almost 100% viable (as shown by a dye exclusion test). During the preparation serum is separated and preserved.

If the lymphocytes are to be used within 72 hours they can be kept at 4°C with the addition of 10% of the patient's own serum to the final 199 medium. Before use they are washed and resuspended in 199, care being taken not to exceed 250*g* in centrifugation.

If, on the other hand, the cells cannot be used within this time, then they may be stored in liquid nitrogen for at least a month without demonstrable loss of immunological activity as studied by the cell electrophoresis method.

To do this, lymphocytes in medium 199 are spun down and resuspended to a concentration of 3×10^6 per ml in freezing medium. This comprises 80% Hank's BSS containing 0·5% lactalbumin hydrolysate (MRC Central Stores) and 20% calf serum (Oxoid), buffered to approximately *p*H 7·2 with 0·035% sodium bicarbonate. This medium is stable at 4°C for up to one month. Immediately before use 10% dimethyl sulphoxide (BDH) is added. In this medium cells may be left up to 24 hours at 4°C. Usually they are dispensed in 1·0 ml hard glass ampoules (Johnsen and Jorgensen) labelled and then heat sealed. These ampoules are left at 4°C for a

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minimum of three hours in order to allow the freezing medium to penetrate cells fully. They may, if convenient, be left overnight. The ampoules are then placed vertically in a 250 ml Pyrex prechilled (4°C) beaker and kept so by means of packing with cotton wool or cellulose wadding, care being taken to see that this material does not intervene between the ampoules and the bottom of the beaker. In practice a Pyrex beaker has been found to be as satisfactory as the BF-5 biological freezer for producing slow and even cooling with the advantage of greater capacity. The beaker is then placed in the top of an LR-35-9 Union carbide liquid nitrogen refrigerator for a minimum of eight hours. Usually it is left there overnight. The frozen ampoules are then transferred to the storage canisters in the liquid nitrogen refrigerator.

When ampoules are removed they are immediately immersed in water at 37°C, either in a water bath or a polypropylene beaker (not glass beaker). There is a danger at this point that a poorly sealed ampoule may explode if liquid phase storage canisters are used and it is advisable to wear goggles and asbestos gloves.

Cells are washed once in medium 199 and viability (as shown by the erythrosin exclusion test) is about 95% after six weeks' storage. Occasionally an ampoule is broken in storage so that it is a good practice to put down three or four.

The cell electrophoresis test (Field and Caspary, 1971; Caspary and Field, 1971) for lymphocyte sensitization is found to be practically unchanged after one month's storage. After six months there is a reduction of about 30%. However, these long-term specimens have been kept under poor conditions in our laboratory in that other lymphocytes were being stored in the same canisters and they all had to be brought out and warmed up each time one was sought. It may well be that under ideal conditions cells may be stored over much longer periods without loss of activity.

Comment

As a means of dealing with the accumulation of specimens on a relatively short-term basis the method described is simple and effective and forms a useful technical adjunct to cell electrophoresis which, even in practised hands, is capable of dealing only with relatively few specimens per session. It has the great advantage over the method outlined by Mangi and Mardiney (1970) that it does not require the presence of the donor's plasma nor the use of anticoagulants. The donor's serum is thus available for other use, whilst the decision to freeze the cells can be made at any point in the preparation.

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