Pulling Lymphocyte Viability for Long-term Transportation of Blood Samples

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Lymphocyte viability in blood samples

For the biological dosimetry, the establishment of lymphocyte cultures may not necessarily be possible at the site, and blood samples have to be carried to the established laboratory for chromosome and/or mutation analysis. In this case, the loss of lymphocyte viability is one of the major problems. The blood samples may be kept for two or three days in the ice cold for a limited use, but upon blood culture the frequency of cells in mitosis is severely reduced. This problem is solved by stimulating the lymphocytes with phytohemagglutinin (PHA) immediately after venipuncture and keeping the cells in the cold so that the lymphocytes are stimulated (cold stimulation) but do not progress toward cell cycle until the cells are warm up to 37 ^{o}C. The lymphocytes may be kept viable for more than 10 days without significant loss of mitotic activity. This makes it possible to bring the blood samples from remote sites for biological dosimetry as well as experimentally irradiated blood samples. For practical application,

1) Prepare a 10 ml sterile tube filled with 5 ml of **Leibovitz's L-15** medium (Gibco BRL) containing 10% fetal bovine serum and 4% rehydrated PHA.

2) Put 5 ml of heparinized blood sample into the tube and mix with the culture medium.

3) Keep the sample tubes in the cold (below 20 ^{o}C). Higher temperature may stimulate the lymphocytes to grow.

4) At this stage, the blood may be kept or mailed without a significant reduction of lymphocyte viability nor mitotic progression.

5) In the laboratory, isolate the lymphocytes, culture in RPMI-1640 medium containing 20% fetal bovine serum and 4% PHA and incubate at 37 ^{o}C for 50 hours. To isolate lymphocytes, slowly put the blood sample onto 3 ml of lymphocyte isolation medium, Picoll-Paque (Pharmacia Biotech), and centrifuge at 300g for 10 mins. Take out the lymphocyte layer and wash the cells with Hanks's salt solution (or phosphate buffered saline) and culture.

Alternatively, cold-stimulated blood samples may be subjected to the whole blood cultures, where 1 ml of blood mixture is mixed with 9 ml of RPMI-1640 medium containing 20% fetal bovine serum and 4% PHA.

6) Treat cultures with colcemid for the last 20-24 hours in the 50 hour culture time, harvest the cells and prepare the chromosome preparations according to the standard procedure.

Selective loss of irradiated lymphocytes and their rescue by cold stimulation

When the lymphocyte populations are homogeneously irradiated or cell inactivation effects are stabilized, loss of lymphocyte viability does not influence the frequency of chromosome aberrations. However, when the irradiation is non-homogeneous and aberration frequency is to be evaluated shortly after irradiation, the cell viability greatly influence the overall aberration frequency. This may be due to the selective loss or inactivation of exposed lymphocytes before stimulation. After *in vitro* irradiation of whole blood with moderate doses of X-rays, delay of culture initiation does not significantly influence the frequency of chromosome aberration irrespective of the loss of viability during the post-irradiation incubation with or without cold stimulation. However, after irradiation with α-particles, the aberration frequency decreases with time of holding without stimulation while cold-stimulated lymphocytes are viable for long time and the aberration frequency stays rather constant. This indicates that the cells receiving hits by α-particles are selectively killed but the cold stimulation rescues those cells from the interphase death. However, the Throtrast patients, where the circulating lymphocytes are receiving hits by α-particles, aberration frequencies are rather stable whether the blood are kept unstimulated or stimulated (Fig. A).

Application to chromosome aberration analysis in the exposed populations

Blood samples were collected from 30 residents who have been living in the vicinity of Semiparatinsk nuclear test sites in Kazakhstan. The blood samples were cold stimulated at sites and transferred by airplane to Radiation Biology Center, Kyoto University for chromosome analysis. The duration between blood sampling and culture varied from 10 to 17 days. An aliquot of cold-stimulated blood sample was used for chromosome analysis and the other was further sent to Radiation Effects Research Foundation in Hiroshima for glycopholin A (GPA) and T-cell receptor (TCR) mutation assay by Dr. S. Kyoizumi and Dr. T. Seyama. For chromosome analysis, the lymphocytes were separated as described and cultured in the presence of PHA for 50 hours. All cultures yielded high frequency of cells in mitosis. In each sample, 500 cells were scored for the types and frequencies of chromosome aberrations. The frequencies of dicentrics and rings are shown in Figure B. Most of the samples showed the aberration frequencies comparable to those of control persons, but some showed apparently high frequency of aberrations.