Letter to the Editor

Improved efficiency of EBV transformation of B-lymphocytes

We read with interest the recent paper by Oh *et al.* (2003) entitled 'An efficient method for the rapid establishment of Epstein-Barr virus immortalization of human B lymphocytes'.

Our experience of routine B-lymphocyte immortalization using Epstein-Barr virus (EBV) at the Welsh Transplantation and Immunogenetics Laboratory extends over 8 years. In our recent study involving the processing of 230 whole blood samples (Bass *et al.* 2004) we reported a transformation success rate of 98.7% using lymphocytes prepared under sterile conditions and 70.4% using cells prepared in a non-sterile environment.

The following information may be useful to Dr Oh and colleagues and to those undertaking routine EBV immortalization of B-lymphocytes.

In addition to initial lymphocyte sterility we consider that three fundamental factors significantly improve transformation success:

- 1 Using high titre viral supernatant from B95-8 marmoset cells (we use a titre of 10^{-5}) and preserving its potency until required by storage in the vapour phase of liquid nitrogen. Cryopreservation in this way is vital as transformation success is compromised when EBV supernatant is stored at 4 °C.
- 2 Using whole blood taken into acid citrate dextrose (ACD) anti-coagulant. This prolongs lymphocyte viability, thereby allowing lymphocytes to be isolated and cryopreserved from whole blood up to 72 h old. This also means that transformations can be efficiently carried out on batches of frozen lymphocytes.
- 3 Using a minimum of 10^6 lymphocytes for each transformation – as lower lymphocyte numbers severely compromises transformation success.

We also consider that a repeat infection with EBV is unnecessary as:

- 1 After a single infection with EBV, microscopic clumps of immortalized B-lymphocytes are normally visible within 3–5 days.
- 2 Lymphocytes from 5 ml of whole blood produce approximately 40×10^6 immortalized B-lymphocytes in approximately 28 days. This is a sufficient number of cells to cryopreserve 10×1 ml ampoules of B-cell line at a concentration of 4×10^6 cells/ml.

Careful adherence to the methods and procedures detailed and referenced in Bass *et al.* (2004) has virtually guaranteed (98.7%) us successful EBV transformation from good quality, sterile ACD whole blood samples. These procedures would greatly simplify the method of H.-M. Oh and colleagues and should also improve their transformation efficiency and success rate.

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REFERENCES

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