A single lymphocyte culture for fragile X induction and prometaphase chromosome analysis

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Abstract

A single lymphocyte culture system is described which produces both reliable fragile X expression and elongated chromosomes for prometaphase analysis. This system, which is based on that described by Wheeler and Roberts in 1987, involves the deoxycytidine release of a thymidine block. Eight fragile X positive subjects had an average expression level of 26%, with a range of 12% to 45%, using the thymidine/deoxycytidine protocol. This was comparable to the levels obtained in parallel cultures treated with thymidine alone or fluorodeoxyuridine.

Several techniques have been developed to produce elongated or prometaphase chromosomes for cytogenetic analysis, and in the last few years the use of an excess thymidine (300 mg/l) block to induce synchrony in lymphocyte cultures has become one of the standard techniques.^{1,2} Thymidine is added the day before harvest to block the cell cycle at S phase and the block is released the following morning either by removal of the thymidine or addition of deoxycytidine.³

Sutherland *et al*⁴ showed that similar levels of thymidine can be used to induce expression of some chromosome fragile sites, in particular fra(X)(q27), the fragile site associated with Martin-Bell syndrome.⁵

The ability to use a single culture system to produce both reliable fragile X expression and elongated chromosomes would clearly be of advantage to a diagnostic cytogenetic laboratory.

Over a period of one year we have been using cultures treated with excess thymidine or fluorodeoxyuridine⁶ for the induction of fragile sites and a third culture treated with a thymidine block released

by deoxycytidine to produce elongated chromosomes from appropriate diagnostic referrals to the laboratory. The levels of fragile X expression induced by all three culture regimens have been compared.

Methods

(A) THYMIDINE INDUCTION OF FRAGILE SITES (THY)

Lymphocyte cultures were established by adding 0.2 ml of heparinised whole blood to 5 ml of RPMI 1640 culture medium supplemented with 10% fetal calf serum and containing penicillin (50 IU/ml), streptomycin (50 µg/ml), L-glutamine (1.4 mmol/l), and phytohaemagglutinin (0.05 ml Gibco M form). The cultures were incubated at 37°C for 72 or 96 hours before harvesting (as convenient for the laboratory). Thymidine (300 mg/l) was added 24 hours and colcemid (0.5 µg/ml) one hour before harvesting by using standard procedures.²

(B) THYMIDINE BLOCK, DEOXYCYTIDINE RELEASE (THY/DOC)

Lymphocyte cultures were established as for method (A). Thymidine (300 mg/l) was added and 24 hours later the block was released by adding deoxycytidine (4.54 mg/l). After a further four hours 15 minutes incubation, colcemid (0.5 µg/ml) was added for 15 minutes and the cultures harvested as for method (A).

(C) FLUORODEOXYURIDINE INDUCTION OF FRAGILE SITES (FDU)

Lymphocyte cultures were established as for method (A) but using TC199 culture medium with 5% fetal calf serum. The incubation and harvesting procedures were also as for method (A) except for the addition of fluorodeoxyuridine (0.1 µmol/l) instead of thymidine.

Control data were obtained from 36 patients screened for fragile X syndrome during the same period who were referred to the laboratory because of developmental delay.

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Received for publication 15 April 1991.

Revised version accepted for publication 11 June 1991.

Expression of fragile X. For each patient the number of cells showing fragile X expression is shown as a proportion of the total number of cells examined from each culture.

Family	Patient	Sex	Age	Cultures					
				THY/DOC		THY		FDU	
				No	%	No	%	No	%
A	1	M	3	9/25	36	6/25	24	7/25	28
B	2	M	3	9/30	30	5/20	25	5/20	25
C	3	F	15	6/40	15	3/22	14	2/18	11
C	4	F	43	8/40	20	3/20	15	1/20	5
C	5	M	18	9/20	45	4/40	10	—	—
D	6	M	4	7/30	23	11/30	37	—	—
D	7	F	30	3/25	12	2/25	8	4/25	16
E	8	M	16	9/25	36	9/25	36	8/25	32
Totals				60/235	26	43/207	21	27/133	20
Range of expression levels				12%–45%		8%–37%		5%–32%	
Controls									
10 females, aged 1 to 11 years				0/118		0/419		0/357	
26 males, aged 0 to 10 years				0/211		3/1165		1/1052	

THY/DOC = thymidine block with deoxycytidine release as described in method (B). THY = unreleased thymidine treatment as described in method (A). FDU = fluorodeoxyuridine treatment as described in method (C).

Results

The expression levels of the fragile X chromosome observed in eight patients from five families are shown in the table. In all eight patients the fragile X expression level for the THY/DOC was similar to those of the other methods used, and in six out of the eight patients the expression level was the highest. This was also reflected in the combined results showing an average expression level of 26% for the THY/DOC cultures compared to 21% for the THY cultures and 20% for the FDU cultures. The THY/DOC cultures showed the highest range of expression levels from 12% (patient 7) to 45% (patient 5) compared with 8% to 37% for the THY cultures and 5% to 32% for the FDU cultures.

The fragile X chromosome was not observed in 329 cells examined from the THY/DOC cultures from 36 control patients. Although these data are limited, they are comparable with the control data from the THY and FDU cultures.

A good mitotic index of cells with elongated chromosomes for prometaphase analysis was obtained from all eight patients.

Discussion

Sutherland *et al.*⁴ showed that an excess of thymidine (300 mg/l) can be used to induce fragile X expression, but that when thymidine synchronised cultures were released from the block by removing the thymidine, the level of expression of the fragile X chromosome was less than one third of the level observed in other fragile X induction regimens. Preliminary unpublished data of our own are consistent with this observation.

Sutherland *et al.*⁴ also showed that the addition of deoxycytidine suppressed the thymidine induced expression of the fragile X chromosome if the concentration of deoxycytidine was high (300 mg/l); however, a lower concentration of deoxycytidine (100 mg/l) only suppressed the expression of the fragile X very slightly.

To produce elongated chromosomes the concentration of deoxycytidine used by Wheeler and Roberts³ to release the thymidine block is much less than the concentrations used by Sutherland *et al.*⁴ Our results show that such low concentrations of deoxycytidine may not significantly affect induction of fragile X expression by thymidine.

Therefore, these preliminary results suggest that the deoxycytidine release of thymidine blocked lymphocyte cultures can be a reliable method of both inducing fragile X expression and producing elongated chromosomes for diagnostic purposes.

Using the THY/DOC protocol would allow a laboratory to use a routine single culture for most referrals for analysis of lymphocyte chromosomes with implications for saving both time and resources. This would also allow the retrospective analysis of cases for fragile X expression without the need for additional samples.

The authors thank all the staff of the Regional Cytogenetics Unit, Birmingham Maternity Hospital for their contributions to these cases, and the clinicians for the referral of their patients.

1 Viegas-Pequignot E, Dutrillaux B. Une methode simple pour obtenir des prophases et des prometaphases. *Ann Genet (Paris)* 1978;21:122–5.

- 2 Watt JL, Stephens GS. Lymphocyte culture for chromosome analysis. In: Rooney DE, Czepulkowski BH, eds. *Human cytogenetics: a practical approach*. 1st ed. Oxford: IRL Press, 1986:39–55.
- 3 Wheeler RF, Roberts SH. An improved lymphocyte culture technique: deoxycytidine release of a thymidine block and use of a constant humidity chamber for slide making. *J Med Genet* 1987;24:113–5.
- 4 Sutherland GR, Baker E, Fratini A. Excess thymidine induces folate sensitive fragile sites. *Am J Med Genet* 1985;22:433–43.
- 5 Nussbaum RL, Ledbetter DH. Fragile X syndrome: a unique mutation in man. *Annu Rev Genet* 1986;20:109–45.
- 6 Glover TW. FUDR induction of the X chromosome fragile site: evidence for the mechanism of folic acid and thymidine inhibition. *Am J Hum Genet* 1981;33:234–42.