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Maturation of Human Peripheral Blood Leukemic Cells in Short-Term Culture

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A. Summary

This work is a continuation of earlier studies in this laboratory (Palú et al. 1979) in which two populations of cryopreserved acute myelogenous leukaemia (AML) cells were whown to undergo progressive maturation in vitro. Twelve other populations of AML cells have since been studied and three distinct patterns of in vitro behaviour have been observed:

1. Cells which did not mature.
2. Cells which matured to the polymorph series and
3. Cells which matured to the macrophage series.

Six populations of AML cells were studied both before and after cryopreservation to demonstrate that exposure to the cryopreservative agent dimethylsulphoxide (DMSO) does not influence the patterns of maturation observed. The effect of thioproline and prostaglandins A1 and A2 on maturation was also studied. It is too early to say whether any correlation between the prognosis of the AML patients and the maturation pattern of their AML cells can be established.

B. Materials and Methods

The AML cells were collected using an NCI/IBM continuous flow blood cell separator (Powles et al. 1974), cryopreserved (Chapuis et al. 1977), and stored in the vapour phase of liquid N_2 below −150°C.

Ampoules of AML cells were thawed rapidly in a 37°C water bath as required and diluted dropwise with 20 ml culture medium. The culture medium used throughout the procedure was RPMI 1640 (Gibco) with 25mM Hepes, 100 units/ml penicillin, 100 ng/ml streptomycin, 600 mg/l L-glutamine and 15% V/V foetal calf serum (FCS). After three min centrifugation at 400 g the cell pellet was resuspended in 10 ml culture medium and layered onto an equal volume of a sodium metrizoate-Ficoll density gradient (Lymphoprep. Nyegaard). The cell suspension was separated at 400 g at the interface for 15 min. The leucocytes lighter than 1.077 g/ml were collected at the interface and washed twice. The viable cells were counted in a haemocytometer by method of Trypan blue exclusion.

Cultures were established in 35 mm tissue culture dishes (Corning 2.500) at a concentration of 5×10^6 or 10^7 viable cells in 3 ml culture medium. The cultures were incubated at 37°C in a moist atmosphere containing 5% CO_2. Every two to three days 1 ml medium was exchanged by gentle aspiration for 1 ml fresh culture medium.

The following tests, selected from those used by Palú et al. (1979), were applied at regular intervals during the 2 to 3 week culture period:

1. Cell proliferation: An estimation was made by using a haemocytometer to count a 1/4 dilution of the non-adherent cell suspension in trypan blue. The adherent cells were lysed by the method of Currie and Hedley (1977) and the nuclei counted;
2. Morphology and cytochemistry: Slides of the non-adherent cultured cells were fixed and stained with Geimsa for morphology purposes and for the enzymes non-specific esterase (NSE) and chloracetate esterase (CAE) to show monocyte and polymorph differentiation, respectively (Li et al. 1973). Adherent cells were fixed and stained directly in the culture dishes; and
3. Fc receptors: The percentage of EA rosette-forming cells was estimated by the technique of Frøland and Wisløff (1976).

C. Results

Figure 1 shows a typical example of each of the three different patterns of maturation seen in the AML cell cultures. Three populations of

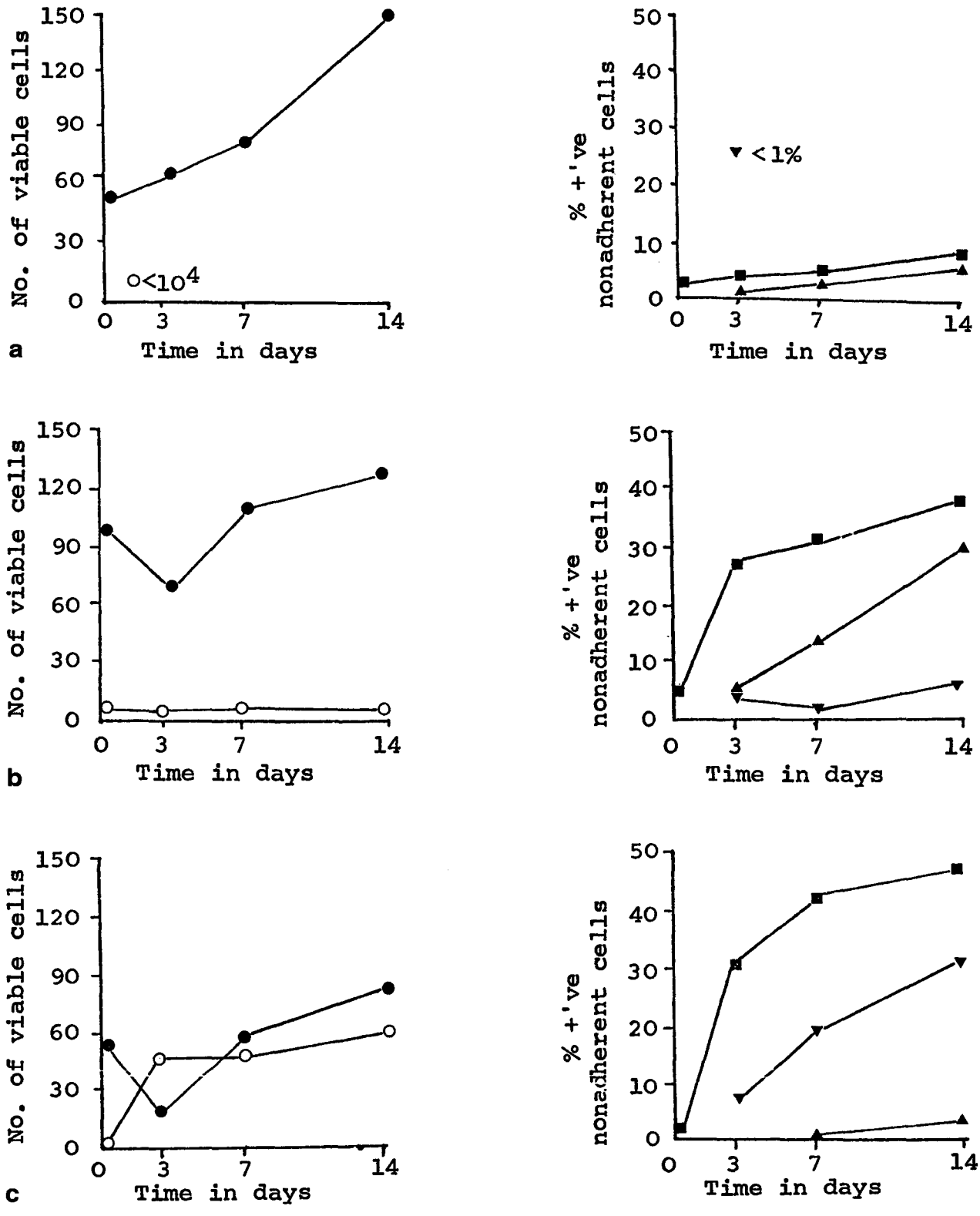

Fig. 1a–c. A typical example of the growth and maturation behaviour of each of the three types observed in vitro. **a** No maturation; **b** maturation to polymorphs; **c** maturation to macrophages. ● non-adherent cells $\times 10^5$. ○ adherent cells $\times 10^4$. ■ Fc+'ve cells. ▲ CAE+'ve cells. ▼NSE+'ve cells.

cells did not mature, although they proliferated in vitro. These three populations had very few adherent cells and the non-adherent cells had very low percentages of Fc positive, NSE positive and CAE positive cells. Six populations showed progressive maturation to the polymorph series in which the fraction of the adherent and the non-adherent cells which were positive for Fc and CAE increased, whilst the percentage of NSE positive cells remained low throughout the culture period. Five populations showed progressive maturation towards

the macrophage series in which the proportion of non-adherent cells which were positive for Fc and NSE increased and more than 90% of the adherent cells were NSE positive. Both adherent and non-adherent cells had low percentages of CAE positive cells.

Six populations of cells were cultured prior to cryopreservation before and after the addition of 5% DMSO and subsequently cultured after cryopreservation to see whether the maturation pattern of the cells was altered in the presence of DMSO. No difference in maturation pattern was seen in any of the three types of pattern described.

Figure 2 shows the effect of prostaglandins A1 and A2 on the percentage of CAE positive cells in the cells of one patient (J.B.) which matured to the polymorph series when cultured in the absence of prostaglandins A1 and A2. The figure illustrates that increasing the amounts of prostaglandins A1 and A2 increased the percentage of CAE positive cells seen. The prostaglandins raised the percentage of Fc positive (i.e. EA rosetting) cells by day 12 to 60% as compared to 40% in the FCS control cultures. The numbers of adherent and non-adherent cells were unaffected by the prostaglandins. No significant effect was seen in the cultures containing thioproline. None of the three chemical agents tested had any effect on the one non-maturing population CP in which they were studied.

Table 1 summarises the clinical state of the 14 patients whose AML cells were studied in vitro. As yet it is too early to determine whether the in vitro behaviour of the cells is related to the prognosis of the patients.

References

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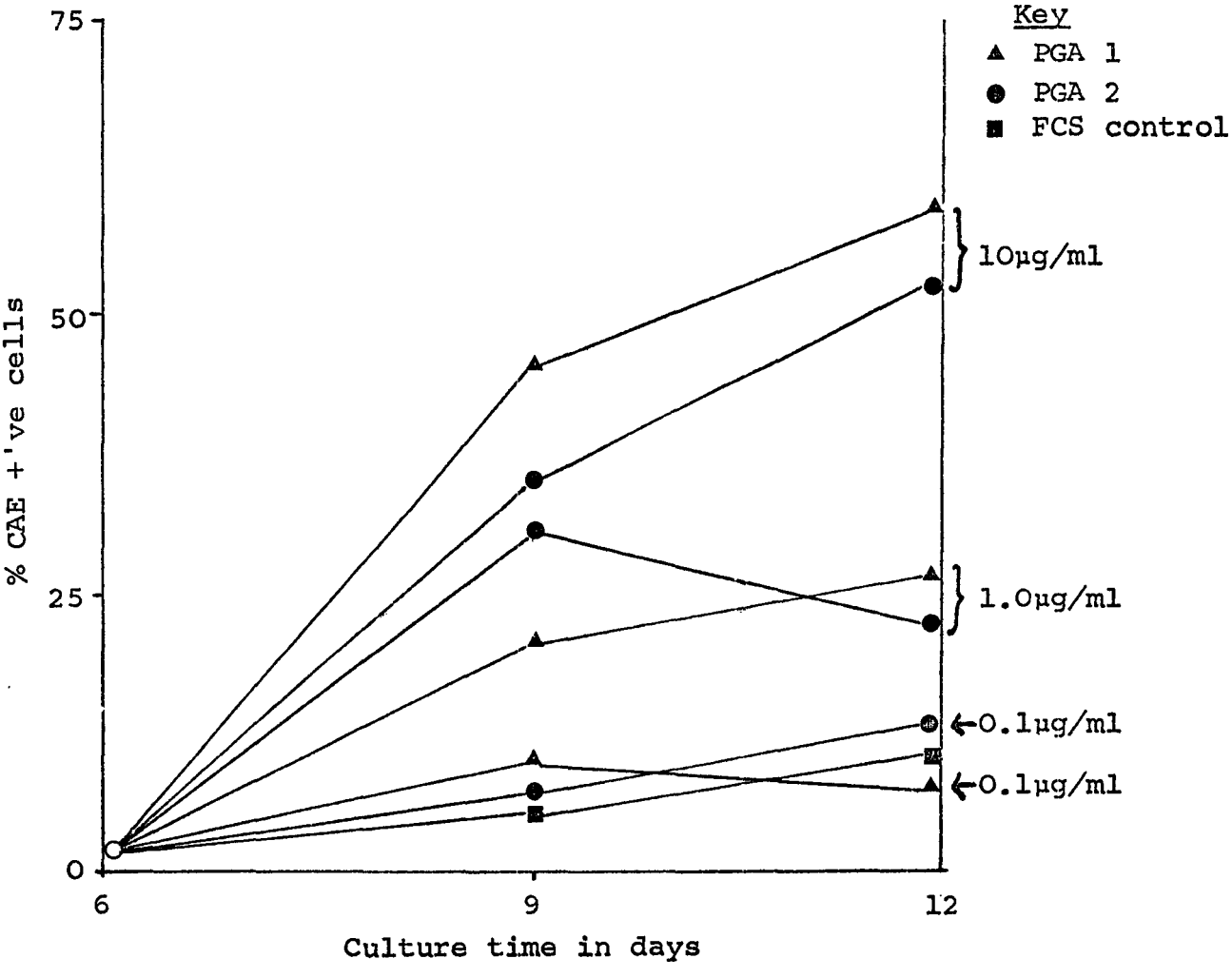

Fig. 2. Percentage of CAE+'ve non-adherent cells in patient J.B. cultured cells in 3 concentrations of prostaglandins A1 and A2

Table 1. Clinical status of patients whose AML cells were studied in vitro

Maturation Patient type	Diagnosis	Obtained 1st remission	Length of 1st remission	Survival time
No maturation				
E.K.	AMML	no	–	5 months
C.P.	AMbL	yes	6 months	9 months
V.S.	AMML	not yet	–	5 weeks→
Maturation to polymorphs				
J.B.	AMbL	yes	9 months	24 months
D.H.	AMoL	no	–	3 months
G.J.	AMbL	yes	2 months→	3 months→
C.Pr.	AMbL	yes	15 months	27 months
J.S.	AMML	not yet	–	4 months→
E.W.	AMML	yes	15monts	25 months
Maturation to macrophages				
R.H.	AMoL	yes	2 weeks→	8 weeks→
I.I.	AMbL	not yet	–	7 months→
F.L.	AMML	yes	4 weeks→	2 months→
C.S.	AMML	no	–	1 month
J.W.	AMb	yes	8 months	17 months

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