Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

Granulocyte-Macrophage Progenitor Cell Proliferation and Differentiation

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Cells from both the blood and marrow produce factors which stimulate proliferation and differentiation of granulocyte-macrophage (GM) progenitors in vitro. These two processes are revealed in semi-solid agar cultures by progressively increasing clone size and cellular maturity, and for normal human progenitor cells seem intricately linked. The results of this study suggest that the two processes are, at least in part, individually regulated.

A. Materials and Methods

The culture technique was that of Pike and Robinson [5]. Overlayers contained $0.4–1.0 \times 10^5$ neutrophil-depleted, non-adherent marrow cells (six donors) or $2–3 \times 10^5$ liquid nitrogen stored GM progenitors (from two patients with chronic myeloid leukaemia). Differentiation was assessed from days 3–7 in two ways. First, by morphological and cytochemical analysis of clone cells in dehydrated overlayer gels, using either Wright's stain or a stain for non-specific and chloracetate esterases based on the method of Li et al. [4]. Secondly, since differentiation transitions in the GM progenitor pathway are associated with a progressive loss in proliferative capacity [1], we tested recloning ability, using resuspended cells from two to four pooled day 4 or 7 overlayers, cultured for a further 4–7 days on fresh, blood leucocyte, feeder layers.

Two IgG monoclonal antibodies, OKT3 and OKT11a (Ortho Labs) and one IgM, MBG6 (gift of Dr. A. McMichael) were used with complement lysis to remove populations of T-lymphocytes from marrow or peripheral blood cells [3]. The remaining cells were then incorporated into feeder layers (10^6 cells). Control feeder cells were exposed to antibody or complement alone or were untreated. The $OKT4^+$ and $OKT8^+$ cells used in selective replacement experiments were prepared from blood lymphocytes using affinity columns [6]. The bone marrow endogenous CSA assay and units system are described by Francis et al. [2].

B. Results

Increasing clone cell maturity was followed from day 3–7 of culture by measuring the decline in the proportion of clones containing blasts/undifferentiated cells and the increase in the proportion of clones containing mature neutrophils and macrophages. Depletion of feeder layer T cells using OKT3/MBG6 and complement delayed this process (Fig. 1). Day 3–7 cultures on these feeders showed comparatively immature clones (assessed by morphological/cytochemical characteristics of cells in 200–300 consecutive clones). This difference was obtained irrespective of whether the comparison was made with (a) untreated feeder layers (eight experiments), (b) feeder layers depleted of $OKT11a^+$ cells (11 experiments), or (c) controls: complement alone, antibody alone, complement and irrelevant ascites (ten experiments). Peripheral blood and marrow cell feeders with both fresh or stored target cells showed comparable results and are not shown separately.

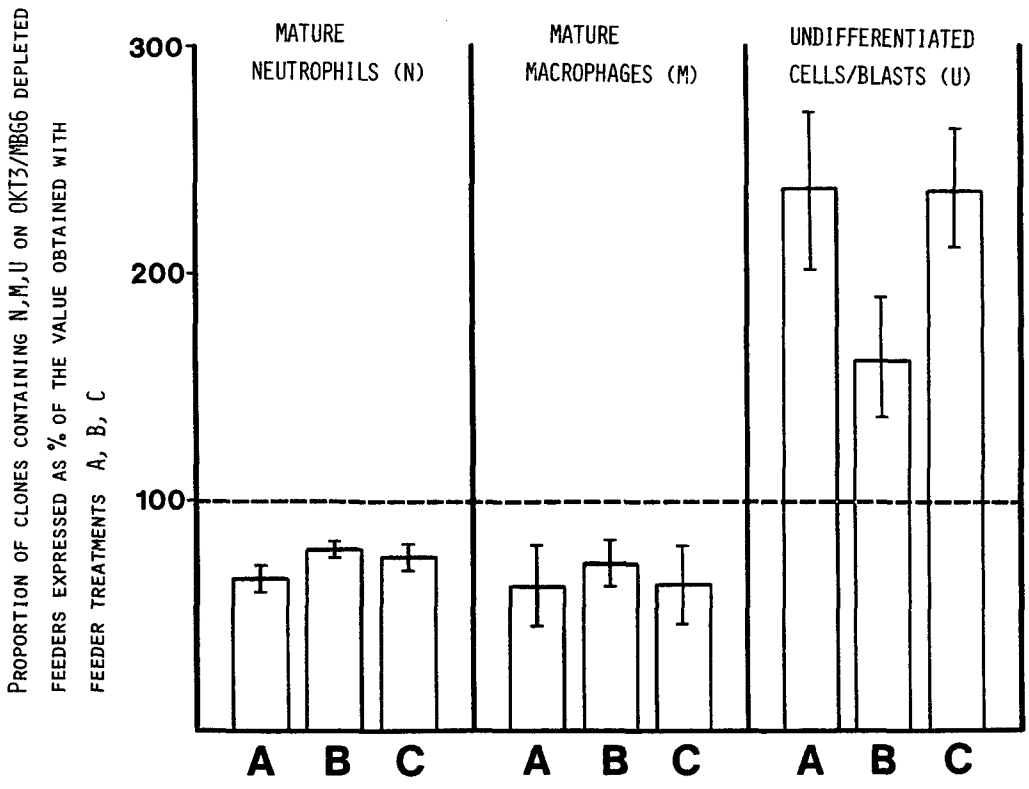

Fig. 1. Depletion of feeder layer T cells, using OKT3/MBG6 and complement, produced relatively immature clone cells compared with feeder layer treatments A, B and C (see text). The differences in the proportions of clones containing N, M and U were statistically significant (Wilcoxon matched pairs signed ranks test, $P < 0.001$)

Serial studies showed that clone numbers were stable from day 3–7 and there was no difference between OKT3/MBG6 depleted, OKT11a depleted and untreated feeder layers (151±5, 151±6 and 157±6 clones/10^4 marrow cells respectively, means±SE of five daily values).

Recloning experiments showed that there was an 89%±28% increase in the number of secondary clones per primary clone when primary cultures were grown on OKT3/MBG6-depleted rather than OKT11a-depleted feeders (the OKT11a-treated controls showed 1ry/2ry clone ratios of 0.65, 0.13 and 0.25, means of three experiments. The secondary clones contained both granulocytes and/or macrophages. Some were large colonies of over 200 cells by day 7 of secondary culture.

Figure 2 shows the effect of replacing the removed OKT3/MBG6$^+$ cells with either OKT4$^+$- or OKT8$^+$-enriched populations. The OKT8$^+$ but not the OKT4$^+$ cells tended to reverse the depression of clone maturity index caused by OKT3/MBG6 depletion. This reversal was only partial; complete reversal would have required an increase of maturity index to 489% of the mean OKT3/MBG6-depleted index value (1.97).

Bone marrow endogenous CSA assays showed that whereas OKT3/MBG6 depletion produced a qualitative change in the stimulus, OKT11a depletion produced a quantitative change. Untreated controls produced 16.0±5.4 units of CSA/10^5 bone marrow cells (mean±SE of three samples). OKT11a-depleted marrow produced increased CSA, 231±37% of control values in three assays. All other treatments produced no significant change in CSA level. At 10^6 cells/feeder all marrows would have provided CSA in excess, stimulating maximal clone formation (> 100 units of CSA).

C. Discussion

The results showed that when feeder layers were depleted of T-lymphocytes bearing the OKT3/MBG6 antigen(s) there was a significant change in the rate of differen-

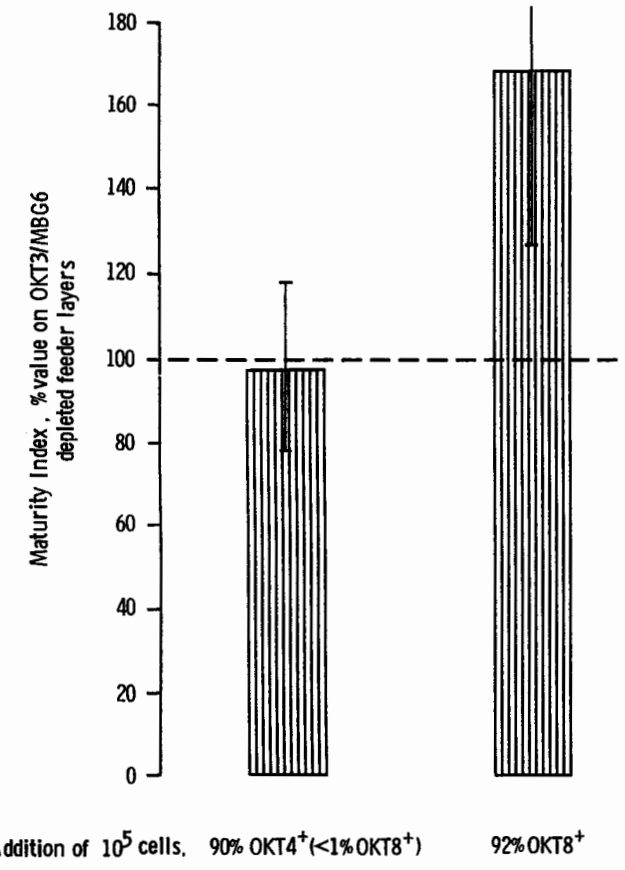

Fig. 2. The effect on clone maturity of replacing removed OKT3/MBG6$^+$ cells with 10^5 OKT4$^+$-enriched or OKT8$^+$-enriched cells. The maturity index = % clones containing mature neutrophils + % clones containing mature macrophages ÷ % clones containing blasts. Means ± SE of three experiments; 100% = 4.4, 1.1 and 0.38 respectively

tiation in the developing GM clones in the overlayer. Replacement of the removed OKT3/MBG6$^+$ cells with OKT8$^+$ cells partially reversed this effect, suggesting that the cells responsible for the selective effect on differentiation have the phenotype OKT8$^+$OKT3$^+$MBG6$^+$. The observation that there was no difference in the number of clones formed on OKT3/MBG6-depleted feeders suggests that the differences observed did not merely reflect recruitment of an additional slowly differentiating clonogenic cell subset, or the failure of clone formation by the most rapidly differentiating cells.

The recloning experiments suggest that the morphological and cytochemical immaturity of the clone cell is accompanied by functional immaturity, with delay in the loss of proliferative capacity which normally accompanies differentiation in the GM progenitor pathway [1].

The contrasting qualitative and quantitative changes in CSA produced by removing OKT3/MBG6$^+$ or OKT11a$^+$ cells presumably result from minority populations bearing only OKT11a (4%–5%) or OKT3/MBG6 (< 1%) antigens.

References

1. Francis GE et al. (1981) Stem Cells 1:124–139
2. Francis GE et al. (1981) Exp Hematol 9:32–45
3. Granger S et al. (1982) Brit J Haematol 50:367–374
4. Li CY et al. (1973) J Histochem Cytochem 21:1–12
5. Pike BL, Robinson WA (1970) J Cell Physiol 76:77–84
6. Tidman N et al. (1981) Clin Exp Immunol 45:457–467