Journal of Supramolecular Structure 13:513–524 (1980)
Control of Cellular Division and Development: Part B: 67–78

The Role of Cells and Their Products in the Regulation of In Vitro Stem Cell Proliferation and Granulocyte Development

T. M. Dexter, E. Spooncer, D. Toksoz, and L. G. Lajtha

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England

In long-term marrow cultures haemopoiesis can be maintained in vitro for up to 6 months. Critical analysis of the cell populations produced has shown that the stem cells and their committed progeny have characteristics in common with the corresponding cell types in vivo. The maintenance of haemopoiesis in vitro is associated with the development of an appropriate inductive environment provided by bone marrow derived adherent cells. Analysis of the interactions between environmental and haemopoietic cells has been facilitated by the development of in vitro systems reproducing the naturally occurring genetic environmental defects and other systems where the development of a competent inductive environment shows a dependency upon corticosteroid hormones. Investigations have shown that stem cell proliferation may be controlled by production of opposing activities, one stimulatory for DNA synthesis, the other inhibitory. A model is proposed whereby modulation in the production of these factors is determined by the physical presence of stem cells in a proposed cellular milieu, within the adherent layer. The adherent layer, apart from acting at the level of stem cell proliferation, can also modify the response of differentiating cells (eg, GM-CFC) to exogenous stimulatory activities. Addition of GM-CSF or of CSF-antiserum has no effect on haemopoiesis in long-term cultures.

Key words: long-term marrow cultures, cell-cell interactions, microenvironment, stem cell, proliferation modulators, GM-CFC, differentiation

In multicellular organisms where cells are collected into discrete, specialized functional units, a continual loss of cells occurs through natural wastage (= death). In some circumstances, when loss of relatively large numbers of cells may not be detrimental to reproductive survival of the species (eg, brain tissue), little or no replacement occurs. In most systems, however, the loss of cells must be compensated for by the production of more cells also of a specialised nature. Since the majority of specialised cells are so-called "end-cells" – where specialisation has occurred concomitantly with a loss of reproductive capacity – the continual production of these cell lineages necessitates a population of self-renewing stem cells [1]. The control of proliferation and differentiation (the process whereby commit-

Received March 3, 1980; accepted June 12, 1980.

0091-7419/80/1304-0513$02.30

ment occurs) of stem cells remains an overriding question in biology, and is being approached on many levels. Of the regenerating systems available for study in mammals, the haemopoietic system offers one of the best means to approach this difficult problem, since the products of differentiation (the committed progenitor cells) can be readily cloned in vitro and their mature (specialised) progeny recognised and examined by a variety of techniques [2].

In the mouse, the pluripotential haemopoietic stem cells can be recognised by their ability to form colonies in the spleens of potentially lethally irradiated mice [3]. These spleen colony forming cells (CFU-S) give rise to cells of restricted potential which nonetheless possess extensive proliferative ability. The majority, if not all of these restricted cells can be cloned in vitro in the presence of appropriate stimulatory factors. Included in this category are GM-CFC (granulocyte/macrophage progenitors) which form colonies of mature end cells when cultured in the presence of colony stimulating factor (GM-CSF) [4, 5], early and late erythroid progenitor cells (BFU-E, CFU-E) which respond to erythropoietin [6,7] and other factors [8, 9], megakaryocyte colony forming cells [10], and lymphoid clonogenic populations [11].

We have been approaching the problem of regulation of haemopoietic cell proliferation and differentiation using an in vitro system where haemopoiesis is maintained for up to 6 months. In such cultures there is proliferation of CFU-S and production of granulocytic, erythroid, megakaryocytic, and lymphoid progenitor cells [12–14]. The maintenance of haemopoiesis in vitro depends upon the prior establishment of a suitable population of bone marrow derived adherent cells, which we have suggested provides the inductive environment necessary for haemopoiesis to occur [14, 15]. In this article some aspects of these long-term cultures are briefly reviewed as well as some recent data from experiments designed to study the role of the cellular environment in determination.

ESTABLISHING LONG-TERM CULTURES

Detailed methodology has been described elsewhere [13, 14]. Briefly, the contents of a single femur from young adult mice are inoculated into Falcon 25cm^2 culture flasks containing 10 ml of Fischers' medium supplemented with horse serum and antibiotics [13]. Occasionally foetal calf serum supplemented with hydrocortisone [16] was used. Over a 2–3 week interval an adherent layer containing endothelial-like cells, fat cells, some fibroblasts, and macrophages is formed [14, 15]. Onto this established adherent layer are then inoculated 5×10^6–10^7 syngeneic, semi-allogeneic, or allogeneic [17, 18] bone marrow cells. This inoculation constitutes 0 time for the cultures, which are then fed by removal of half the growth medium and addition of an equal volume of fresh medium (normally weekly). The growth medium removed contains cells in suspensions which can be assayed for CFU-S or the variety of committed progenitor cells as described previously [13, 14].

Two aspects are worthy of note: a) haemopoiesis is improved if the cultures are maintained at 33°C [14] and b) when horse serum or foetal calf serum supplemented with hydrocortisone is used, the second re-inoculum of fresh marrow cells is usually unnecessary [16, 19] ie, the kinetics of the system are such that an appropriate "stromal" environment is formed before the stem cells have been lost through differentiation or death.

Successful long-term marrow cultures can be established from a majority of mouse strains tested, although some strains (notably H-2^K) do demonstrate poor growth in vitro [17, 20].

HAEMOPOIESIS IN LONG-TERM CULTURES

Stem cells and their committed progeny can be produced for up to 30 weeks. In all aspects so far studied, the cells produced appear to have characteristics and functions comparable with the equivalent cell population in vivo (Table I). While the studies are by no means exhaustive, they strengthen our contention that the long-term cultures are representative of haemopoiesis in vivo and provide a suitable model system for investigating the role of humoral factors and cell-cell interactions.

The Role of the Environment

Several lines of research suggest that the adherent cells play a role in haemopoiesis. Some evidence is indirect – such as the finding that prolonged stem cell maintenance does not occur in siliconised culture flasks (where cell attachment is prevented) even when supplemented with conditioned medium from long-term cultures, nor on spleen cell adherent layers, embryonic fibroblasts, soft agar cultures, or diffusion chambers ([14] and Dexter, T.M. unpublished). Such studies indicate that not only are specific cellular interactions important but that close interactions between specific populations play a key role. Compelling evidence for this hypothesis comes from our studies with genetically determined haemopoietic defects [27]. The macrocytic anaemia seen in W/W^v mice is caused by an intrinsic defect in the stem cell population. In $S1/S1^d$ mice on the other hand, a similar macrocytic anaemia is related to a defective inductive haemopoietic environment [28].

TABLE I. Haemopoiesis in Long-Term Cultures

Cells produced	Characteristics	Comparison with in vivo	Reference
CFU-S	pluripotent		13,14,21
	reconstitution ability		14,21
	seeding efficiency		Toksoz, D, Dexter, TM (unpub.)
	biophysical	normal	Eliason, J, Dexter, TM (unpub.)
	response to steroids		22
	karyotype (indirect)		Scott, D, Dexter, TM (unpub.)
GM-CFC	GM maturation		14,23
	amplification		this paper
	sedimentation velocity	normal	23
	GM-CSF response		14,23
	karyotype		Dexter, TM (unpub.)
	response to cytotoxic agents		Dexter, TM (unpub.)
BFU-E	response to BPA and erythropoietin amplification	normal	24,25
Lymphoid precursors	development in vivo	normal	21
Mature cells	GM receptors and function	normal	23,26
	platelet production		

Further, the anaemia in W/W^v mice can be corrected by infusion of stem cells from S1/S1^d mice, whereas the corresponding anaemia in S1/S1^d mice can only be alleviated by transplantation of a competent inductive environment (eg, W/W^v spleen tissue). Analysis of these two strains using long-term marrow cultures [27] has demonstrated that the defects can be reproduced and "overcome" in the culture system (Table II).

Defective environments, unable to maintain haemopoiesis, are also produced when marrow cells are cultured in most batches of FCS (Table III). Adherent layers produced in FCS-supplemented cultures are striking for their absence of fat cell generation, although endothelial cells and macrophages are still being produced. In these cultures, haemopoiesis rapidly declines. However, when the growth medium is supplemented with 10^{-6} or 10^{-7}M hydrocortisone [16], extensive fat cell generation is seen, and the cultures will support stem cell proliferation and differentiation for several months. If hydrocortisone addition is delayed for two weeks after establishing the adherent layer, fat cells do not develop, although all other cell types are present. This indicates that the lipid synthesising cells are a minority population distinct from most fibroblasts and endothelial cells. They are probably depleted from the cultures if hydrocortisone addition is delayed. The effect of hydrocortisone addition is rapid and dramatic; but while the biological consequence of haemopoietic stimulation seems to be related to fat cell development, the underlying biochemical mechanism is obscure. We have recently initiated a biochemical analysis of defective environmental milieu using these two systems; the genetically determined lesions (S1/S1^d) and the FCS/hydrocortisone supplemented cultures. The result will be reported elsewhere, but it seems clear that consistent differences in cell matrix synthesis can be detected in competent vs defective adherent layers.

The Control of Stem Cell Proliferation

We have shown that when long-term cultures are fed by total depopulation (ie, removal of all the growth medium and replacement with fresh medium), the number of CFU-S within the adherent layer remains fairly constant while, at the same time, large numbers of CFU-S are released into the growth medium [19, 22]. Over many weeks, the adherent layer acts as the major site of stem cell production. When the proliferative activity of CFU-S was measured (using a ^{3}HTdR suicide technique), it was found that within a few hours post-feeding, the CFU-S both in the adherent layer and remaining in suspension, had entered into an actively cycling state ($>$ 35% kill with ^{3}HTdR). This high cycling was maintained for 3–4 days, then subsequently declined to a low cycling level ($<$ 10% kill) [29]. At the same time, the CFU-C were maintained in a uniformly high cycling state. When the cultures were re-fed after seven days, a similar wave of CFU-S cycling occurred. In further work [30] we have shown that this oscillation in CFU-S cycling is intimately associated with the production of specific CFU-S proliferative inhibitory and stimulatory molecules, of the type described by Lord and others [31–34]. A summary of these data is shown in Table IV. The growth medium removed from long-term cultures 1 and 7 days, post re-feeding was centrifuged, and the cell pellet assayed for CFU-S numbers and cycling using established procedures [29, 30]. The cell-free growth medium was then fractionated into various molecular weight components using Amicon filtration and the different extracts were freeze dried and stored as described previously [31, 32]. In other systems it has been shown that fraction III (30–50,000 daltons) contains the CFU-S stimulatory activity and fraction IV (50–100,000 daltons) contains the inhibitory material [31, 32]. The extracts obtained from long-term cultures were then assayed for inhibitory or stimulatory effects on CFU-S cycling. Fraction III from 1d post feeding cultures increased cycling

TABLE II. Use of Genetically Defective Mice in Analysis of Haemopoietic Environments

In Vivo

W/W^v: normal environment, *defective CFU-S*

S1/S1^d: *defective environment,* normal CFU-S

In Vitro

adherent layer	re-inoculum	haemopoiesis
W/W^v	W/W^v	declines
S1/S1^d	S1/S1^d	declines
S1/S1^d	W/W^v	declines
W/W^v	S1/S1^d	SUSTAINED

The reader is referred to [27] for detailed analysis of this system.

TABLE III. Effect of Foetal Calf Serum on the Haemopoietic Environment

Group	Adherent cells produced	Total cells (× 10^5)		CFU-S	
		2 wk	20 wk	2 wk	20 wk
FCS alone	endothelial macrophage fibroblast	4.0	2.0	< 20	0
FCS + 10^{-6}M hc (0 time)	endothelial macrophage fibroblast FAT CELLS	67.0	48.0	600	720
FCS (2 weeks)[a] + 10^{-6}M hc	endothelial macrophage fibroblast	5.6	4.0	< 50	0

hc, hydrocortisone; FCS, foetal calf serum (20% in Fischers' medium).

[a]The cultures were grown in FCS alone for two weeks prior to addition of hc.

of quiescent stem cells from < 10% to greater than 35% using a minimal dose of 50 μg/ml. However, the same fraction from 7d post feeding medium had a similar effect on CFU-S cycling, but only using a dose of 100 μg/ml, ie, the relative concentration or specific activity of III was higher in 1d post feed cultures (where CFU-S were actively cycling) than in 7d post feed cultures (where CFU-S are non-cycling). Similarly, fraction IV from 1d post feeding medium showed an inhibitory effect on CFU-S cycle at a dose of 200 μg/ml, whereas the corresponding fraction from 7d post feeding medium was effective at a significantly lower dose, ie, the presence of quiescent CFU-S in the 7d feeding cultures is associated with a relative increase in the inhibitory activity. These data and others [30] showing that addition of exogenous fraction III or IV directly to the long term cultures has a dramatic effect upon CFU-S cycling, with no detectable cytotoxic effects, suggest that stem cell proliferation is determined by modulation of the production of these opposing activities.

What then is the stimulus for production of these factors? The simplest interpretation is that the depopulation of the stem cells (as a consequence of feeding the cultures) is somehow 'recognised' by the producer cells, which respond by producing excess stimulator until the status quo is restored (when stimulation is switched off and inhibitor levels increase). However, work in our laboratory has shown that when the long-term cultures were fed with fresh medium containing *excess* stem cells (eg, adding back the original cultured CFU-S), CFU-S proliferation followed the same pattern as that seen with addition of fresh medium alone. Even when the cultures were re-fed with old medium (removed from 7d post feeding cultures) and excess CFU-S, a similar pattern of stem cell cycling was observed (Toksoz, D. unpublished data). In further investigations of this problem we then found that simple mechanical agitation of the culture bottles was sufficient to induce stem cell cycling. Long-term cultures, 8 days post re-feeding (where CFU-S are non-cycling), were shaken vigorously for 2–3 seconds and returned to the incubator. No further manipulations such as feeding were employed. At various times later, cultures were assayed for cycling activity of CFU-S and for the activities of fraction III and IV in the growth medium. The results (Fig. 1) show that within a few hours after shaking, actively cycling CFU-S are found within both the adherent layer and in the growth medium. The overall profile was similar to that seen in re-fed cultures [29]. When the culture medium was examined for the presence of fractions III and IV, we found that within a few hours after shaking the specific activity of stimulatory material in fraction III had significantly increased, while the activity of IV had decreased (Table V). A mechanical disturbance of the long-term cultures, therefore, resulted in alteration in the levels of fraction III versus IV leading to CFU-S cycle activation.

It seems probable that a major effect of shaking will be on the spatial organisation of cells and on the intercell concentration of "signal" materials. One obvious effect would be to interfere with the cell/cell relationships between the 'non-adherent' and the adherent cell fraction. Because the 'non-adherent' cell population contains the majority of CFU-S [19, 22], it follows that the interaction between CFU-S and the adherent cell population will be temporarily disturbed. Since it is also known that the stimulator and inhibitor producing cells are adherent, light density, non CFU-S cells [35, 36] the interaction between CFU-S and these cells will also be disrupted. Based upon these assumptions, it is possible to formulate several models as to the control of CFU-S proliferation in this system, based upon interactions between the stem cell and inhibitor (I)/stimulator (S) producing cells. Two possibilities are shown in Figure 2. In both models (A and B, Fig. 2) we assume that local stem cell milieux exist defined on the basis of cellular organisation. Within this milieu, CFU-S exert either a positive influence on the production of inhibitory activity (A) or a negative influence on the production of stimulatory molecules (B). When the stem cells are displaced from this milieu (either by differentiation, migration, cytotoxicity (death) or *mechanical disturbance),* the local milieu is disturbed. In model A, the positive influence on inhibitor cell production is negated, while the concentration of stimulator remains the same, ie, in this example there is no need for modulation of stimulator production. In model B, displacement of CFU-S leads to a release of the stimulator-producing cell and more stimulator is produced, although inhibitor levels remain constant. In both cases (A and B) the net result will be cycling of stem cells – presumably resulting in the production of more CFU-S. Passively or actively, some of these will eventually occupy the determinative environmental milieu, and the respective feedback systems will be resumed. Naturally, other more complex models can be formulated, ranging from a dual effect of CFU-S on both the inhibitor and stimulator producing cells to interactions between the inhibitor and stimulator pro-

TABLE IV. Production of Factors Affecting CFU-S Proliferation in Long-Term Cultures

	Source of extract			
	1D post feeding (high cycling CFU-S)		7D post feeding (low cycling CFU-S)	
μg/ml	% ^{3}HTdR kill III[a]	% ^{3}HTdR kill IV[a]	% ^{3}HTdR kill III[a]	% ^{3}HTdR kill IV[a]
0	5 ± 2	33 ± 4	2 ± 3	39 ± 3
10	7 ± 4	31 ± 3	ND	36 ± 5
30	12 ± 7	ND	6 ± 2	ND
50	22 ± 2	36 ± 9	2 ± 1	23 ± 7
100	28 ± 4	18 ± 7	24 ± 6	6 ± 2
200	ND	9 ± 5	32 ± 5	9 ± 6

Note: Fractions II and V were also assayed for stimulatory/exhibitory and were consistently negative.
[a]Dose in μg/ml required to produce stimulation (III) or inhibition (IV) of CFU-S cycling as measured by ^{3}HTdR suicide technique. Fraction IV was assayed using target populations of regenerating bone marrow (cycling CFU-S) and Fraction III activity assayed on normal bone marrow (low cycling CFU-S). The rationale and methodology have been detailed elsewhere [20, 30, 32].

TABLE V. CFU-S Proliferation Factors Produced in Mechanically Agitated Cultures

	Source of extract			
	7D post feeding		1D post agitation[a]	
μg/ml	% ^{3}HTdR kill III[b]	% ^{3}HTdR kill IV[b]	% ^{3}HTdR kill III[b]	% ^{3}HTdR kill IV[b]
0	2 ± 3	39 ± 3	6 ± 4	35 ± 4
50	2 ± 1	23 ± 7	27 ± 4	21 ± 6
100	24 ± 6	6 ± 2	35 ± 2	21 ± 3
200	32 ± 5	9 ± 6	33 ± 6	4 ± 9

[a]7D post feeding cultures shaken for 2–3 seconds and the conditioned medium collected 24 hours later.
[b]As in legend to Table IV.

ducing cells themselves. However, to be useful a model needs to be open to experimental analysis. Using the long-term cultures this is indeed possible in that a) we can measure *absolute* amounts as well as specific activity of factors produced after 7 days and 7 days plus 1 day post-shaking to determine whether there is modulation of the production of only one component or both and b) using competent and defective environmental mileux deficient in haemopoiesis (such as the W/W^v versus S1/S1^d), we can determine the importance of the physical presence of stem cells in factor production. Indeed, preliminary results from our laboratory indicate that S1/S1^d adherent layers are defective in their capacity to produce fraction III (the CFU-S cycle stimulator) (Toksoz, D.; Dexter, T.M.; and Lord, B.I. unpublished)

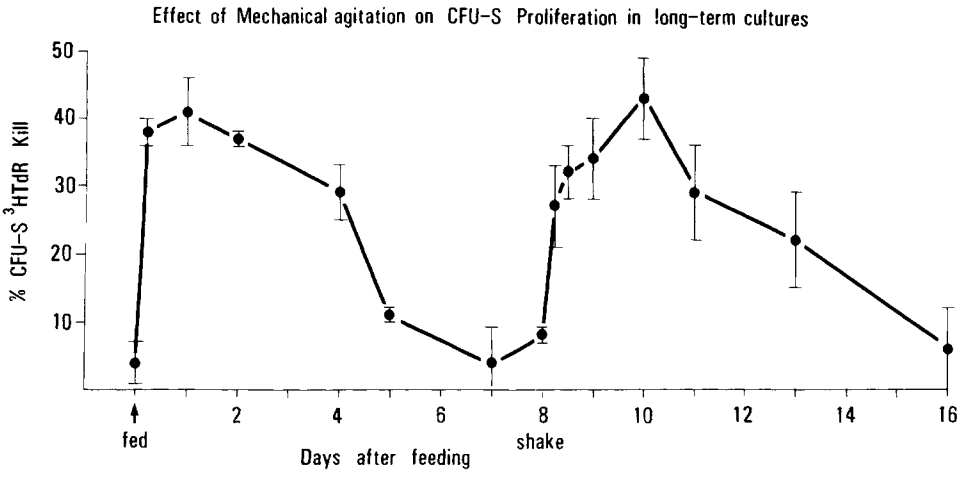

Fig. 1. Long-term marrow cultures were maintained on a demi-depopulation feeding regime for three weeks. At various times after the third feeding, 2 or 3 cultures were used to assay the cycling status of CFU-S (measured using a ^{3}HTdR suiciding technique [20, 30, 32]. Eight days after feeding, a group of cultures was vigorously shaken for 2–3 seconds, returned to the incubator, and cycling status followed. CFU-S recovered from the adherent cells and from the overlying growth medium showed a similar cycling profile. Results (pooled adherent and non-adherent CFU-S data) represent the mean ± S.E. In control non-shaken cultures, CFU-S cycling remained at a minimal level (< 10% ^{3}HTdR kill) between 6 and 16 days post feeding.

The Regulation of Granulopoiesis

Differentiation of stem cells into GM-CFC and the subsequent maturation and amplification of these cells results in the extensive production of mature granulocytes. Analysis of these cell populations in a typical long-term culture is shown in Figure 3A. GM-CFC in the growth medium are maintained at a level of 5×10^3–10^4 per culture for at least 16 weeks. At the same time, between 2.5×10^6–9×10^6 maturing granulocytes (predominantly mature neutrophils) are being produced. In simple terms, this means that 500–1,000 mature cells are produced for every one GM-CFC (ie, a 10 cycle "worth" amplification) from the GM-CFC population) – a figure agreeing well with the colony size generated in soft-agar systems [2, 37]. However, in soft-gel media the development of GM-colonies is absolutely dependent upon the continual presence of stimulating factors (GM-CSF). In the absence of GM-CSF, colonies do not form [2]. Furthermore, analysis in vivo of granulocyte/macrophage populations in cyclic neutropenias has shown a correlation with GM-CSF levels, based upon which a physiological role of GM-CSF has been postulated [38]. Of some considerable interest, therefore, was the finding that long-term marrow cultures do *not* contain biodetectable GM-CSF – nor is GM-CSF inhibitory material produced [14, 23]. Since the GM-CFC in long-term cultures also require GM-CSF *when plated in soft agar* and show the same dose response relationships as normal marrow GM-CFC [14, 23], the granulopoiesis observed in long-term cultures is not a reflection of autonomous GM-CFC which have acquired GM-CSF independence. Some other, more subtle mechanism presumably is responsible for the sustained granulopoiesis.

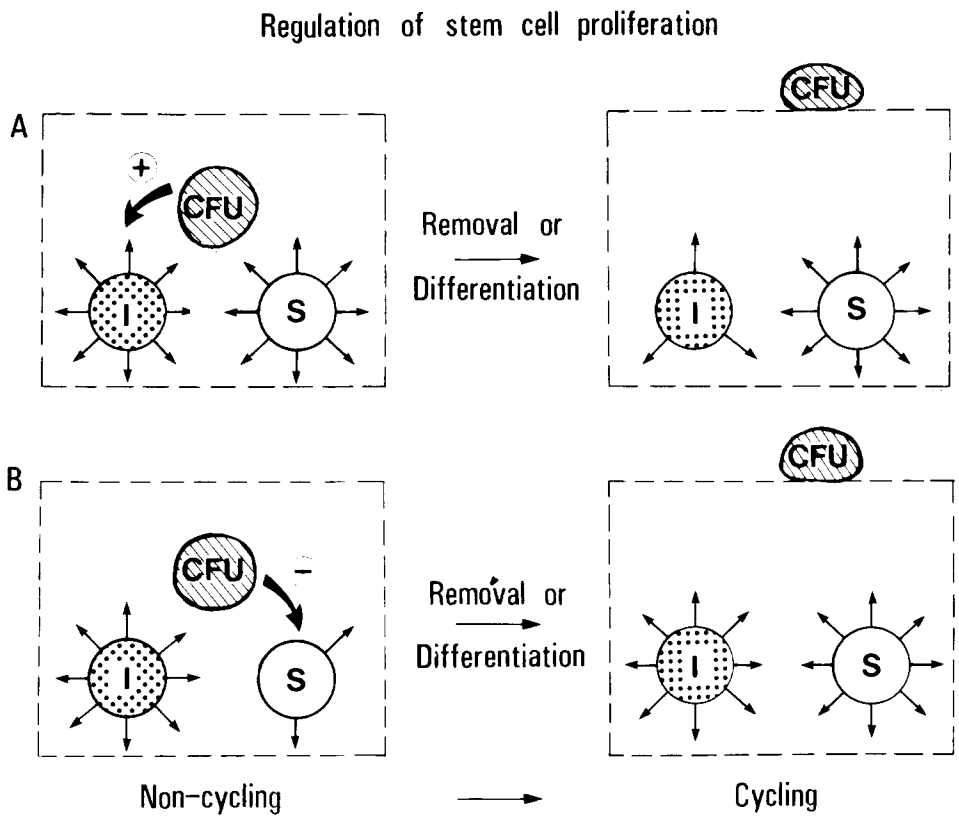

Fig. 2. CFU, pluripotent stem cell; S, stimulator (Fraction III) producing cell; 1, inhibition (Fraction IV) producing cell. Dashed area represents a hypothetical local cellular environment. While I and S are shown as distinct cells, it is possible that they represent different metabolic states of the same cell population.

In previous work we have shown that when conditioned media containing GM-CSF were added to long-term cultures, a decline in haemopoiesis was observed in 2–3 weeks [14]. However, in more recent work it has been demonstrated that this decline cannot be attributed to the actions of GM-CSF since, when highly purified GM-CSF preparations were used, no decline was observed [39, 40]. One relevant experiment is shown in Figure 3B. Long-term cultures were treated weekly with pure L-cell GM-CSF (at a dose which induced the development of plateau numbers of colonies in the agar assay system) or with an antiserum raised against L-cell GM-CSF (at a saturating dose) [39]. When compared with untreated controls, we found no significant differences between production of CFU-S, GM-CFC, total nucleated cells (mainly granulocytes), or in the ratio of production of granulocytes to macrophages. A similar result has been reported by others [40] using an alternative GM-CSF source. These results are intriguing in many respects. First, the lack of effect of CSF antiserum is suggestive that at least one species of GM-CSF is not produced in biologically relevant amounts, accessible to antibody binding, in the long-term cultures (since the activity of such CSF presumably would be 'neutralised' by the antiserum).

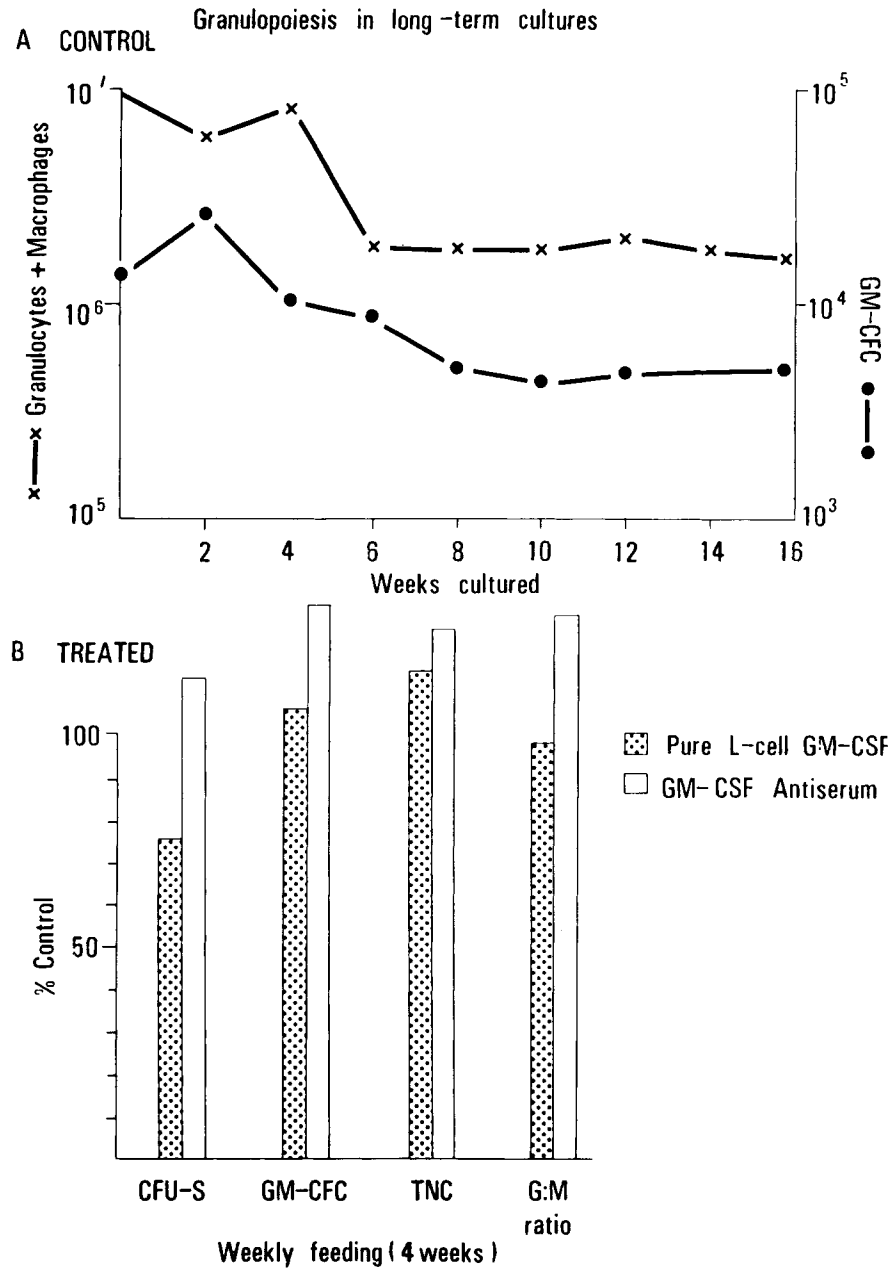

Fig. 3. A) Total cellularity and GM-CFC production in the non-adherent cell fraction of long-term culture. B) Effect of addition of pure L-cell GM-CSF or of an antiserum raised against L-cell GM-CSF on haemopoiesis in long-term cultures (as % untreated controls). The cultures were treated at 0 time with either GM-CSF or antiserum and maintained on a weekly demi-depopulation regime. At each feeding, growth medium containing GM-CSF or antiserum was added. The results show the data after 4 weeks continual maintenance on the reagents indicated [39]. TNC, total nucleated cells; G:M, granulocyte/macrophage ratio.

Secondly, the stimulatory effect of purified GM-CSF on long-term cultured GM-CFC in soft agar and the lack of efect of this material when added *directly* to long term cultures, indicates that GM-CFC development is under strict surveillance in the long-term culture. If this were not the case, it might reasonably have been expected that addition of GM-CSF would lead to an exuberant haemopoiesis following stimulation of the GM-CFC present, and the resulting production of mature cells. Because the major site of production of GM-CFC is from within the adherent layer [19, 22] we speculate that control of GM-CFC development is mediated by intimate interactions between the adherent cells and the responding cell populations. Work in vivo showing that L-cell GM-CSF antiserum has no effect upon marrow haemopoiesis would support this possibility [41]. Whether this means that GM-CSF is not required for GM development in vivo (or in the long-term cultures) or that control is regulated by transmembrane passage of GM-CSF (in amounts which are not biodetectable) remains to be determined.

CONCLUSION

The data presented in this article serve to illustrate the complex cell-cell interactions occurring in long-term marrow cultures. These interactions appear to be operative at all levels of development, from the pluripotent stem cell through the committed progenitor cells and their progeny. Some of these relationships are already being defined in terms of cellular milieu and regulatory molecules produced.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council and the Cancer Research Campaign. T.M.D. is a Fellow of the Cancer Research Campaign.

REFERENCES

1. Lajtha LG: Differentiation 14:23, 1979.
2. Metcalf D: "Hemopoietic Colonies. Recent Results in Cancer Research." Vol 61. New York: Springer-Verlag, 1977.
3. Till JE, McCulloch EA: Radiation Res 14:213, 1961.
4. Bradley TR, Metcalf D: Aust J Exp Biol Med 44:287, 1966.
5. Pluznik DH, Sachs L: J Cell Physiol 66:319, 1965.
6. Iscove NN, Sieber F, Winterhalter KH: In Robinson W (ed): "Haemopoiesis in Culture: Second International Workshop." DHEW publication no (NIH) 74-205, Washington, DC: Government Printing Office 1973, p 44.
7. Stephenson JR, Axelrad AA, McLeod DL, Shreeve MM: Proc Natl Acad Sci USA 68:1542, 1971.
8. Iscove NN, Guilbert L: In Murphy MJ (ed): "In Vitro Aspects of Erythropoiesis." New York: Springer-Verlag, 1978, p 3.
9. Wagemaker G: In Murphy MJ (ed): "In Vitro Aspects of Erythropoiesis." New York: Springer-Verlag, 1978, p 44.
10. Metcalf D, MacDonald HR, Odartchenko N, Sordat B: Proc Natl Acad Sci USA 72:1744, 1975.
11. Metcalf D, Nossal GJV, Warner NL, Miller JFAP, Mandel TE, Layton JE, Gutman GA: J Exp Med 142:1534, 1975.
12. Dexter TM, Lajtha LG: Br J Haematol 28:525, 1974.
13. Dexter TM, Testa NG: In Prescott D (ed): "Methods in Cell Biology." Vol 14, New York: Academic Press, 1976, p 387.
14. Dexter TM, Allen TD, Lajtha LG: J Cell Physiol 91:335, 1977.
15. Allen TD, Dexter TM: Differentiation 6:191, 1976.

16. Greenberger JS: Nature 275:752, 1978.
17. Dexter TM, Moore MAS, Sheridan APC: J Exp Med: 145:1612, 1977.
18. Dexter TM, Spooncer E: Nature 275:135, 1978.
19. Dexter TM, Allen TD, Lajtha LG, Krizsa F, Testa NG, Moore MAS: Cold Spring Harbor Symp Quant Biol 42:63, 1978.
20. Reimann J, Burger H: Exp Hematol 7:45, 1979.
21. Schrader JW, Schrader S: J Exp Med 148:82, 1978.
22. Dexter TM: In Lajtha LG (ed): "Clinics in Hematology." London: Saunders Co, Vol 8:453, 1979.
23. Williams N, Jackson H, Rabellino EM: J Cell Physiol 93:435, 1977.
24. Eliason J, Testa NG, Dexter TM: Nature 281:382, 1979.
25. Gregory CJ, Eaves AC: In Clarkson B, Marks PA, Till JE (eds): "Differentiation of Normal and Neoplastic Hematopoietic Cells." Cold Spring Harbor Symp Quant Biol 42:179, 1978.
26. Williams N, Jackson H, Sheridan APC, Murphy MJ, Elste A, Moore MAS: Blood 51:245, 1978.
27. Dexter TM, Moore MAS: Nature 269:412, 1977.
28. Russell ES, Bernstein SE: In Green EL (ed): "Biology of the Laboratory Mouse." New York: McGraw Hill, 1966, p 351.
29. Dexter TM, Wright EG, Krizsa F, Lajtha LG: Biomedicine 27:344, 1977.
30. Toksöz D, Dexter TM, Lord BI, Wright EG, Lajtha LG: Blood (in press).
31. Lord BI, Mori KJ, Wright EG: Biomedicine 27:223, 1977.
32. Lord BI, Mori KJ, Wright EG, Lajtha LG: Br J Haematol 34:441, 1976.
33. Frindel E, Croizat H, Vassort F: Exp Hematol 4:56, 1976.
34. Frindel E, Guigon M: Exp Hematol 5:74, 1977.
35. Wright EG, Lord BI: Leuk Res 3:15, 1979.
36. Wright EG, Lord BI: Biomedicine 28:156, 1978.
37. Pluznik D, Sachs L: Exp Cell Res 43:553, 1966.
38. Moore MAS, Spitzer G, Metcalf D, Pennington DG: Cyclic neutropenia. Br J Haematol 27:47, 1974.
39. Dexter TM, Shadduck RK: J Cell Physiol 102:279, 1980.
40. Williams N, Burgess A: J Cell Physiol 102:287, 1980.
41. Shadduck RK, Carsten AL, Chikkappa G, Cronkite EP, Gerard E: Proc Soc Exp Biol Med 158:542, 1978.